Iron status, anthropometric status and cognitive performance of black African school children aged 6-11 years in the Klerksdorp area

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May

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"Be strong and courageous, and do the work. Don't be afraid or discouraged by the size of the task, for the LORD God, my God, is with you. He will not fail you or forsake you…"

1 Chronicles 28:20

To my **S**hepherd, **S**avior, **H**elper and **F**riend

To the **O**ne who was, and is and is to come

To the **O**ne whose ways are higher than my ways, and whose thoughts are higher than my thoughts.

Glory be unto **G**od!

By **Y**our mighty power that works within me, I was able to accomplish infinitely more than I would ever have dared to ask or hope for.

May this work reflect **Y**our name and **Y**our name alone, may **Y**our kingdom come, and **Y**our will be done.

Love, Christine

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Ian and **C**harlotte, for your endless love and support, and for the solid foundation you laid for me to stand upon.

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Carla, even though you're the youngest you're my greatest teacher.

My friend,

Zelda, thank you for your constant friendship throughout this challenge and for being the perfect example of dedication.

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Dr **C**ovic, you are my inspiration! It is a privilege to know you, to work with you, and to have you as my supervisor and friend.

ABSTRACT

AIM

Poor iron status and under-nutrition among children are of concern not only in South Africa but worldwide. Both independent and combined associations between poor iron status, under-nutrition and cognitive development and function have been investigated. This mini-dissertation investigated possible associations between iron status indicators, anthropometric nutritional status and cognitive performance in the Beverage Fortified with Micronutrients (BeForMi) study population (black South African children aged 6-11 years in the North-West province of South Africa).

METHODS

The study was cross-sectional and based on the BeForMi study baseline data. Primary school children (n = 414) with the highest serum transferrin receptor (STR) and zinc protoporphyrin (ZnPP) levels were included. Anthropometric z-scores – BMI-for-age (BAZ), height-for-age (HAZ), and weight-for-age (WAZ) – and iron status indicators – haemoglobin (Hb), serum ferritin (SF), STR and ZnPP – were determined. The Kaufman Assessment Battery for Children, Second edition (KABC-II) was used to generate cognitive scores.

RESULTS

Fourteen percent of children were underweight (WAZ ≤ 2 SDs), 12.8% stunted (HAZ ≤ 2 SDs) and 8.4% wasted (BAZ ≤ 2 SDs). Of the children, 7.1% were anaemic (Hb < 11.5 g/dL), 13% iron depleted (Hb ≥ 11.5 g/dL and SF < 12 µg/L) and 2.7% had iron deficiency anaemia (Hb < 11.5 g/dL and SF < 12 µg/L). Low iron stores (SF < 12 µg/L) were observed in 15.7% of the children. Positive correlations were found between SF and WAZ ($r = 0.1$, $p = 0.047$), Hb and HAZ ($r = 0.13$, $p = 0.007$) and WAZ ($r = 0.13$, $p = 0.009$). Positive correlations with small effect sizes were observed between some cognitive scores and z-scores ($p < 0.05$, r-value range 0.10 - 0.24). Negative correlations with small effect sizes were observed for the subtests Triangles and Rover (both subtests on simultaneous processing) with Hb ($p = 0.008$, $r = -0.13$) and SF ($p = 0.04$, $r = -0.1$) respectively. Higher HAZ, WAZ and education level of the head of household were all significantly associated with the likelihood that a child would fall within the upper quartile of Hb values in our study group ($p = 0.036$, $p = 0.032$ and $p = 0.036$ respectively).

CONCLUSION

The results suggested that under-nutrition was positively associated with poor iron status and lower cognitive scores in this study population. Further research, investigating specific effects of poor iron status at different stages of growth and the relationship with cognitive function later in life may help explain the negative correlations observed between current iron status indicators and cognitive scores.

Keywords: cognitive performance, black South African children, stunting, wasting, under-nutrition, iron status

OPSOMMING

DOEL

Swak ysterstatus en ondervoeding onder kinders is nie slegs ’n bekommernis in Suid-Afrika nie, maar ook wêreldwyd. Beide onafhanklike sowel as gesamentlike verwantskappe tussen swak ysterstatus, ondervoeding en kognitiewe ontwikkeling en -funksie is reeds ondersoek. Hierdie skripsie ondersoek moontlike assosiasies tussen ysterstatusindikatore, antropometriese voedingstatus en kognitiewe prestasie in die *“Beverage Fortified with Micronutrients (BeForMi)”* -studiepopulasie (swart Suid-Afrikaanse kinders tussen 6- en 11- jarige ouderdom in die Noordwes Provinsie van Suid-Afrika).

METODE

Die studie het ’n dwarsdeursnit ontwerp gehad en was gegrond op die *BeForMi*-studie se basislyndata. Die insluiting- en uitsluitingskriteria het laerskoolkinders (n = 414) met die hoogste serumtransferrienreseptor (STR)- en serumsinkprotoporfirien (RSP)-vlakke ingesluit. Antropometriese z-tellings – liggaamsmassa-indeks (LMI)-vir-ouderdom (BAZ), lengte-vir-ouderdom (HAZ) en gewig-vir-ouderdom (WAZ) – sowel as ysterstatusindikatore naamlik hemoglobien (Hb), serumferritien (SF), STR en RSP was bepaal. Die *Kaufman Assesserings Battery* vir kinders (tweede uitgawe) was gebruik om die kognitiewe tellings te genereer.

RESULTATE

Veertien persent van die kinders was ondervoed (WAZ ≤ 2 standaardafwykings, SAs), 12.8% het belemmerde groei (HAZ ≤ 2 SAs) en 8.4% (BAZ ≤ 2 SAs) het wegkwyning ondervind. Van die kinders was 7.1% anemies (Hb < 11.5 g/dL), 13% ysteruitgeput (Hb ≥ 11.5 g/dL en SF < 12 µg/L) en 2.7% het ystertekortanemie gehad (Hb < 11.5 g/dL en SF < 12 µg/L). Lae ysterstore (SF < 12 µg/mL) is gevind in 15.7% van die kinders. Positiewe korrelasies is gevind tussen SF en WAZ ($r = 0.1$, $p = 0.047$), Hb en HAZ ($r = 0.13$, $p = 0.007$) en WAZ ($r = 0.13$, $p = 0.009$). Positiewe korrelasies met klein effekgroottes is waargeneem tussen sommige kognitiewe tellings en z-tellings ($p < 0.05$, r-waarde se reikwydte 0.10 - 0.24). Negatiewe korrelasies met klein effekgroottes is waargeneem vir die sub-toetse *Driehoeke* en *Rover* (beide is sub-toetse van gelyktydige verwerking) met Hb ($p = 0.008$, $r = -0.13$) en SF ($p = 0.04$, $r = -0.1$) onderskeidelik. Hoër HAZ, WAZ en opleidingsvlak van die hoof van die huishouding was

betekenisvol geassosieer met die kans dat ’n kind binne die boonste kwartiel van Hb-waardes in die studie- populasie sou val (p = 0.036, p = 0.032 en p = 0.036 onderskeidelik).

GEVOLGTREKKING

Die resultate suggereer dat ondervoeding positief geassosieer was met swak ysterstatus en laer kognitiewe tellings in hierdie studiepopulasie. Verdere navorsing, wat spesifiek ondersoek instel rakende die effek van swak ysterstatus gedurende verskillende fases van groei en die verhouding met kognitiewe funksie op ’n latere stadium in die lewensiklus is nodig voordat die negatiewe korrelasies wat gevind is tussen die huidige ysterstatusindikatore en kognitiewe tellings verklaar sal kan word.

Sleutelwoorde: kognitiewe prestasie, swart Suid-Afrikaanse kinders, verdwerging, wegkwyning, ondervoeding, ysterstatus

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LIST OF ABBREVIATIONS

BAZ/Age	Body Mass Index-for-age z-score
BeForMi	Beverage Fortified with Micronutrients
CEN	Centre of Excellence
CHU	Child Health Unit
CPAS-R	Cognition-Psychomotor Assessment System-Revised
CRP	C-reactive protein
FCDA	Foodstuffs, Cosmetics and Disinfectants Act
GABA	Gamma-Aminobutyric Acid
HAZ	Height-for-age z-score
Hb	Haemoglobin
HH	Household
HST	Health Systems Trust
IDA	Iron deficiency anaemia
IQ	Intelligence Quotient
ISAK	International Standards for Anthropometric Assessment
KABC-II	Kaufman Assessment Battery for Children II
MI	Micronutrient Initiative
MOSBY	Mosby's Dictionary of Medicine, Nursing & Health Professions
MPI	Mental Processing Index
NCHS	National Centre for Health Statistic

NFCS	National Food Consumption Survey
NFCS-FS	National Food Consumption Survey – Fortification Baseline
NFFP	National Food Fortification Programme
NHANES	National Health and Nutritional Examination Survey
NSNP	National School Nutrition Programme
NVI	Non-Verbal Index
NWU	North West University
OMD	Oxford Concise Medical Dictionary
PSC	Public Service Commission
PSNP	Primary School Nutrition Programme
RDA	Recommended Daily Allowance
SAVACG	The South African Vitamin A Consultative Group
SF	Serum Ferritin
STR	Serum Transferrin Receptor
TReNDS	Training & Research for Nutrition and Dietetic Solutions
UNICEF	United Nations Children's Fund
UNU	United Nations University
USD	United States Dollar
WAZ	Weight-for-age z-score
WHO	World Health Organization
WISC-R	Wechsler Intelligence Scales for Children-Revised
ZnPP	Zinc Protoporphyrin

GLOSSARY

Black African children	Black South African children between the age of 6 and 11 years, attending one of the three preselected primary schools.
Anaemia	Defined as a low concentration of hemoglobin in the blood, varies by age and sex.
Anthropometric indicator	An international reference that includes standardized age- and sex-specific growth reference to calculate height-for-age *Z*-scores (Ht/A), weight-for-age *Z*-scores (Wt/A), weight-for-height *Z*-scores (Wt/Ht) and body-mass-index for-age *Z*-scores (BMI/A).
Cognition	High level physiological processes involved in perception, attention, memory, language, problem solving, reasoning, and making decisions.
Cognitive development	The construction of thought processes, including remembering, problem solving, and decision-making beginning in infancy and continuing to change or progressively improve through adolescent and adulthood.
Cognitive function	Intellectual/mental processes involving symbolic operations such as learning, memory, thinking, movement, reasoning, attention and language. Cognitive function can be divided into executive, memory, attention, perception and psychomotor functions and language skills.
Cognitive performance	An expression of a desired result of a learning experience according to specific cognitive tests conducted.
Iron Deficiency Anaemia	The final stage of the development of iron deficiency, when iron stores are exhausted, circulating iron is very low, red cell production is drastically reduced and anaemia develops. Indicated by age appropriate haemoglobin and serum ferritin values.
Iron deficiency	The most significant contributor to the onset of anaemia.

Iron depletion	The amount of storage iron in the liver, spleen and bone marrow progressively decreases and can be detected by a parallel fall in serum ferritin concentration.
Stunting	Height-for-age < -2 standard deviations of the WHO Child Growth Standards median Stunting is the result of long-term nutritional deprivation that reflects on a process of failure to reach linear growth potential.
Underweight	BMI-for-age < -2 standard deviations of the WHO Child Growth Standards median Reflecting on body mass relative to chronological age.
Wasting	Weight-for-height < -2 standard deviations of the WHO Child Growth Standards median Wasting or thinness indicates in most cases a recent and severe process of weight loss, often associated with acute starvation and/or severe disease.

CHAPTER 1: INTRODUCTION

1.1 Background and motivation

Child under-nutrition is highly prevalent in low- and middle-income countries and includes wasting, stunting, underweight and deficiencies in micronutrients such as iron. Globally, 10% of children are estimated to be wasted (Black *et al.,* 2008). Underweight in low- and middle-income countries is estimated at 20%, while in low-income countries alone, the estimation for stunting is 32% (Black *et al.,* 2008). Furthermore, iron deficiency has been reported to be the most prevalent nutritional deficiency in the world (Biesalski & Erhardt, 2007:37).

In South Africa, three national surveys have been conducted over the past 15 years: the 1994 survey by the South African Vitamin A Consultative Group (SAVACG) (SAVACG, 1995), the 1999 National Food Consumption Survey (NFCS) (Labadarios, 1999) and the 2005 National Food Consumption Survey-Fortification Baseline (NFCS-FB) (Kruger *et al.*, 2007). These studies included reports on two public health concerns which are of worldwide importance and which also affect South African children, namely poor nutritional and iron status.

Chronic and acute malnutrition contribute to poor nutritional status among South African children. On a national level, the prevalence of wasting appears to have remained the same, with 9.3% reported in both 1994 and 2005, while stunting rates seem to have declined from 22.9% (SAVACG, 1995) in 1994 to 18% in 2005 (Kruger *et al.*, 2007). Notwithstanding the promising decline in stunting rates, further reduction is desirable. Natural catch-up is not common for children after the age of five years in developing countries and may not be achieved (Branca & Ferrari, 2002), causing a ripple effect on a number of factors, including cognitive function.

Only two of the national surveys reported on iron status (SAVACG, 1994; NFCS-FB, 2005) and these surveys confirmed the magnitude of poor iron status in South African children as a public health concern. When these studies are compared, an increase in the prevalence of anaemia is apparent (21.4 to 27.9%) in children between one and five years of age (SAVACG, 1995; Labadarios & Louw, 2007).

Brain development, especially with regard to the frontal lobe, continues throughout childhood, and nutrition (including micronutrients and macronutrients) is likely to impact cognitive function (Bryan *et al.,* 2004). Cognitive function may be negatively affected through acute malnutrition,

presenting as wasting, with the child being more apathetic, exploring the environment less and being less active (Grantham-McGregor, 1995). Cognitive development, however, may be retarded with chronic under-nutrition, manifested as stunting, and it is possible that change in cognitive function may be less likely to occur with increasing age (McKay *et al*., 1978). Short-term iron deprivation, even without anaemia, presents with clinical signs similar to acute malnutrition, including apathy, irritability, decreased attention, inability to concentrate and memory loss (Bourre, 2006). Furthermore, brain development may be influenced via the function of iron in the brain such as the role of iron in myelination and oligodendrocytes when iron deficiency anaemia is reached (Beard & Connor, 2003).

In 1994, the National School Nutrition Programme (NSNP) was introduced (PSC, 2008:viii) without targeting micronutrient deficiencies *per se*. However, with the National Food Fortification Programme (NFFP) that was legislated in 2003 (Foodstuffs, Cosmetics and Disinfectants Act, no 54/1972) (FCDA, 54/1972), programmes such as the NSNP could possibly influence micronutrient status since the food given to the children at school is based primarily on maize meal and wheat flour, both of which are mandatorily fortified under the NFFP.

In light of the poor iron status reported for South African children, and its possible effect on cognitive function, the Beverage Fortified with Micronutrients (BeForMi) study is an intervention study conducted to investigate the effect of a multi-micronutrient fortified beverage on the micronutrient status and cognitive function of primary school children aged 6 to 11 years and also to investigate possible effects of different formulations of the beverage on the body composition of the learners.

This cross-sectional study of the BeForMi study population at baseline investigated associations between iron status, anthropometric indicators of nutritional status and cognitive performance in South African black primary school children.

1.2 Title

Iron status, anthropometric status and cognitive performance of black African school children aged 6-11 years in the Klerksdorp area.

1.3 Study design

This is a cross-sectional study nested in the parent BeForMi study conducted in the North-West province. To be included, children had to be 6-10 years of age by January 2010 and without any apparent health condition. Children should not have been on medication/supplements that could affect nutritional status. Furthermore, they had to attend one of the three selected schools. The selection criteria (discussed in detail in Chapter 3) were such that children of low iron status were chosen, thereby reducing the representativeness of the population. Children were sorted according to the highest serum transferrin receptor (STR) and, thereafter, zinc protoporphyrin (ZnPP) value. Even though the study population is not truly representative, it presents valuable information for population samples with similar characteristics. Signed parental consent was obtained for each child.

1.4 Aim, objectives and hypothesis

The main aim of this cross-sectional study was to determine whether there were associations between iron status, anthropometric indicators of nutritional status and cognitive performance in the baseline BeForMi study population of black African children aged 6-11 years in the North-West province of South Africa.

In order to address the given aim, the following objectives were set:

1. To determine the following iron status indicators for all the children in the study:
    - Haemoglobin (Hb): (g/dL)
    - Ferritin (SF): (µg/L)
    - STR: (mg/L)
    - ZnPP: (µmol/mol heme)
2. To determine the cognitive performance of the children using the Kaufman Assessment Battery for Children, second edition (KABC-II) (Kaufman & Kaufman, 2004).
3. To determine the anthropometric indicators of height, weight, and body mass index (BMI) z-scores (WAZ, HAZ, BAZ respectively) for all the children, using World Health Organisation (WHO)-anthropometry software (2006).
4. To complete a socio-demographic questionnaire (Addendum A) for each participating child.

This study tested the following hypotheses:

- Iron status of the children in the study population is positively associated with cognitive performance.
- Anthropometric status of the children in the study population is positively associated with cognitive performance.
- Iron status of the children in the study population is positively associated with anthropometric status.

1.5 Research team and authors contribution

Table 1.1 Research team of the BeForMi study 2010

Team member	Institution	Role
Dr Namukolo Covic	TReNDS CEN, NWU Potchefstroom Campus	Project Director: directing all aspects of the project and stakeholder communications; training of cognitive assessors and school assistants
Prof. Johann Jerling	TReNDS CEN, NWU Potchefstroom Campus	Project leader: all aspects of the project. Data base manager
Dr Jane Kvalsvig	Child Development Research Unit Kwazulu Natal	Overseeing cognitive assessor training. Guidance on all aspects of cognitive assessment
Prof. Marius Smuts	TReNDS CEN, NWU Potchefstroom Campus	Intervention process logistical advice
Prof. Salome Kruger	TReNDS CEN, NWU Potchefstroom Campus	Advisory role on body composition and on anthropometric measurements. Processing of dietary data
Sr Chrissie Lessing	TReNDS CEN, NWU Potchefstroom Campus	Blood sampling logistical process and blood sampling; determination of Glycaemic Index
Mrs Mari van Reenen	Statistical Consultation Services, North-West University, Potchefstroom Campus	Statistical guidance and analysis
Mrs Noloyiso Matiwane	TReNDS CEN, NWU Potchefstroom Campus	Assistance in training of field assistants,
Dr Averalda van Graan Dr Hattie Wright	TReNDS CEN, NWU Potchefstroom Campus	Assistance in anthropometric measurements
Mrs Ellenor Rossouw, Dr Seye Onabanjo Dr Wayne Tower Dr Karin Conradie	TReNDS CEN, NWU Potchefstroom Campus	Laboratory analysis
Mr Thabang Phinda	TReNDS CEN, NWU Potchefstroom Campus	Data input Day-to-day project logistics
Mrs Sarie Lee	TReNDS CEN, NWU Potchefstroom Campus	Dietary data input
Miss Christine Taljaard	**TReNDS CEN, NWU Potchefstroom Campus**	**Key role in day-to-day administration of project intervention, assistance in baseline and end anthropometric, biochemical and cognitive measurements and baseline statistical analysis**

CEN, Centre of Excellence; NWU, North-West University TReNDS, Training & Research for Nutrition and Dietetic Solutions

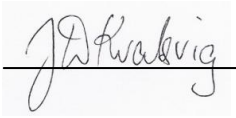
Table 1.2 Level of involvement of the student and authors' contributions to the article to be submitted

Team member	Institution	Role
Miss Christine Taljaard	**TReNDS CEN, NWU, Potchefstroom Campus**	**Full-time M.Sc. student** **Protocol writing** **Statistical analysis** **Article writing**
Dr Namukolo Covic	TReNDS CEN, NWU Potchefstroom Campus	Project Director and Supervisor of M.Sc. dissertation Provided guidance to the student at all stages of the project
Dr Averalda van Graan	TReNDS CEN, NWU Potchefstroom Campus	Co-Supervisor of M.Sc. dissertation Provided guidance to the student at all stages of the project
Prof. Salome Kruger	TReNDS CEN, NWU Potchefstroom Campus	Scientific input
Prof. Marius Smuts	TReNDS CEN, NWU Potchefstroom Campus	Scientific input
Dr Jane Kvalsvig	TReNDS CEN, NWU Potchefstroom Campus	Scientific input
Dr Hattie Wright	TReNDS CEN, NWU Potchefstroom Campus	Scientific input
Mrs Mari van Reenen	TReNDS CEN, NWU Potchefstroom Campus	Statistical guidance and analysis
Prof. Johann Jerling	TReNDS CEN, NWU Potchefstroom Campus	Project leader; scientific input

CEN, Centre of Excellence; NWU, North-West University TReNDS, Training & Research for Nutrition and Dietetic Solutions

Included is a statement from the co-authors, confirming their role in the article and providing permission for the inclusion of the article in this dissertation.

I declare that I have approved the above-mentioned article, that my role in the study, as indicated above, is representative of my actual contribution and that I hereby give my consent that it may be published as part of the M.Sc. dissertation of Miss C. Taljaard.

Dr. N.M Covic	Dr. A van Graan
Prof. H.S Kruger	Dr. H.H Wright
	
Dr. J.D Kvalsvig	Prof. J.C Jerling
Prof. C.M Smuts	Mrs. M van Reenen

1.6 Other study contributors

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School assistants: Thandiwe Ntjengela, Mmamusi Montsho, Itumeleng Ramorou, Puleng Mokoena, Mmasabata Tsolo, Moipone Mbipha, Refilwe Mokgothu, Mpho Paraffin, Nosiphe Marhawule, Matshidiso Mokgosi, Innocent Tshiloane, and Mpho Chauke.

Cognitive assessors: Victoria Nobatana, Gladys Couter, Joseph Dithipe, Lerato Kenosi, Nonasonto Mngxongo, Thabang Phinda, Lydia Mogapi, Alinah Tlhale as well as Namapolise Mildred Thomas who helped with administration of children.

To Sr Chrissie Lessing and her team who conducted all the blood sampling, the logistics of the process, and assisted with baseline and end measurements, a heartfelt thanks for all the work done.

1.7 Structure of this dissertation

This dissertation is presented in article format. The technical aspects of the thesis, with the exception of Chapter 3, follow the guidelines in the postgraduate manual of the North-West University (font Arial, size 11). For Chapter 3, the authors' guidelines of the Public Health Nutrition journal have been used (font Times New Roman, size 12) (Watling, 2008). The decimal system has been used for numbering, except for Chapter 3 where headings are given without numbering.

Following the introductory chapter (Chapter 1) is the literature review (Chapter 2). In this chapter, available published literature has been reviewed with regard to the iron status of South African children. The different levels of iron status are explained and the possible effect of iron status on cognitive function is discussed. Attention is drawn particularly to the possible relationship between iron and cognitive function, including the role of iron in the nervous system. There is a brief discussion of the micronutrients zinc and iodine with regard to their possible effect on cognition, and also of micronutrient interactions and cognitive function. Chapter 2 concludes with a reflection on the NSNP and the possible influence of food fortification on the micronutrient status of primary school children.

Chapter 3 is an article titled "Iron status, anthropometric status and cognitive performance of black African school children aged 6-11 years in the Klerksdorp area". This article is based on cross-sectional baseline data of the BeForMi study population. Referencing follows the Vancouver reference style, as directed by authors' guidelines for the targeted journal, Public Health Nutrition. To provide sufficient information in areas such as the method section, the word limit of the article has not been adhered to, and further word reduction will be made for publication purposes. A copy of the instructions for contributors has been included in addendum B and the informed consent form is in Addendum C.

In the last chapter (Chapter 4), the main findings have been summarised and some recommendations for further work have been made.

A combined reference list for chapter 1, 2 and 4 has been compiled, followed by the addenda.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Iron deficiency is the most prevalent nutritional deficiency worldwide (Biesalski & Erhardt, 2007:37). The impact of iron deficiency on economic cost (UNICEF/UNU/WHO/MI, 1999:1) and human health (WHO, 2008:1) is massive, but until recently, has been almost unrecognised. An increase in the burden on health systems, in reduced adult productivity and in poor school performance are some of the consequences of iron deficiency (UNICEF/UNU/WHO/MI, 1999:1). The World Health Organisation (WHO) (2008:12) has reported that anaemia is a global public health problem, with one in four people being affected. The prevalence of anaemia in African preschool children was reported to be 67.6% in 2008, placing Africa as one of the two WHO regions with the highest risk.

In 2008, almost one third of women and children in South Africa were reported to be anaemic, based on the 2005 National Food Consumption Survey – Fortification Baseline (NFCS-FB) (Labadarios & Louw, 2007:454). The survey results showed that in 2005, 6% of South African children between the ages of one and nine years were iron deficient.

Table 2.1 presents the results of two large South African surveys that reported on iron status. The South African Vitamin A Consultative Group (SAVACG) included 4494 children between the ages of 6 and 71 months in their study in 1994 (SAVACG, 1995) and Labadarios and Louw (2007:447) reported on the iron status of 1730 children between the ages of one and nine years in the NFCS-FB of 2005. When the data of the above-mentioned studies are compared, one can concur with Swart *et al.* (2008) that the iron status of South African children seems to be deteriorating.

In 1999, the National Food Consumption Survey (NFCS) included children between the ages of one and nine years (Labadarios, 2000). Data were collected by means of qualitative food frequency questionnaires and 24-hour recall questionnaires, and the results showed that 25% to 37% and 41% to 63% of children at national level consumed less than half the recommended intake of 10mg/day iron, respectively (Labadarios, 2000). The Survey of 1999 reports only the dietary intake of iron and not the biochemical iron status of the children. Therefore, it is not possible to compare iron status results of the SAVACG (1995) study and the NFCS (1999).

From the information given, it would appear that more needs to be done to address the dietary iron intake as well as the iron status of South African children. This is of great importance because of the impact that poor iron status may have on the health as well as on the cognitive performance of children.

Table 2.1 Proportion (%) of children with anaemia, iron depletion and iron deficiency in South Africa based on the SAVACG-1994 (SAVACG, 1995) and NFCS-FB-2005 (Labadarios, 2007)

Indicator	Year	Percentage of children
Anaemia		
Hb <11g/dL; children ≤60 months	1994	21.4
Hb<11.5g/dL; children > 60 months	6 months to 6 years	
	2005	27.9
	1-9 years	
Iron depletion		
Hb≥11g/dL; children ≤60 months or	1994	4.8
Hb≥11.5 g/dL; children >60 months and ferritin	6 months to 6 years	
<12µg/L	2005	7.8
	1-5 years	
	2005	5.7
	1-9 years	
Iron deficiency		
Hb<11g/dL; children ≤60 months or	1994	5.0
Hb<11.5g/dL; children >60 months and ferritin	6 months to 6 years	
<12µg/L	2005	11.3
	1-5 years	
	2005	7.6
	1-9 years	

Hb, Haemoglobin

The aim of this literature review is to give an overview of the possible role that iron status and iron intake may have on cognitive function. Information on zinc and iodine will also briefly be presented. Finally, the possible contribution of the National School Nutrition Programme (NSNP) to the micronutrient status of South African children will be discussed. Even though it is well-known that micronutrient deficiencies have the largest impact on child and brain development during pregnancy and the first 2 years of life (Sachdev *et al.,* 2005), less information at later stages of life are available. Furthermore, less information exist on the extent to which some effects of nutrient deficiencies on cognitive function could be reversed with supplementation in pre-primary and primary school aged children. Therefore this literature

review will focus mostly on pre-school and primary school children, for the intervention study itself was conducted in children between the age of six and eleven years.

2.2 The role of nutrition in cognitive development

Food provides the raw materials needed not only for energy requirements, but also for growth and development. It is not surprising that good nutrition would be associated with a positive effect on cognitive development and function.

Good health and nutrition have been positively associated with less school grade repetition, less absenteeism, more grades completed and better performances on test scores (Behrman, 1996). Each of the factors mentioned can have an influence on cognitive performance, and research has probed questions regarding the relationship between food, health and education (McKay *et al*., 1978; Taras, 2005).

Protein-energy malnutrition has been reported as having significant negative effects on tests of cognitive function in preschool and school-aged children (Leslie & Jamison, 1990; Behrman, 1996). Stathis *et al*. (1999) conducted a prospective study involving 2986 children from birth up to five years of age to determine the extent to which childhood short stature was associated with cognitive and behavioural problems (comprising social, attention and thought). Results suggested a small but significant association between height and cognitive function (Stathis *et al.,* 1999). Similar results are reported by Kordas *et al.* (2004) with first-grade Mexican children (n=724), where a significant association between stature and certain cognitive outcomes was found. When factors such as lead, nutrition, and psychosocial confounders were taken into account for the regression analysis, the association of stature with the cognitive outcomes weakened (Kordas *et al.,* 2004). As would be expected, the stature of children forms part of several factors that could be associated with cognitive function, and these factors should not be overlooked.

When nutritional deprivation reaches a degree that is severe enough to cause stunting, cognitive development may also be retarded (McKay *et al*., 1978). It has been reported that this state of retardation may be less likely to alter with increasing age (McKay *et al*., 1978). Schoenthaler and Bier (1999) reported that there was no evidence that children who were healthy and well-fed could benefit from acute micronutrient supplementation and, that therefore, cognitive performance *per se* would not be enhanced (Bellisle, 2004). However, in population groups that consumed a diet characterised by low vitamins and minerals, supplementation could reverse the effects of poor nutritional status on certain cognitive performance indicators

(Bellisle, 2004; Schoenthaler & Bier, 1999). It is, however, not clear which cognitive indicator may be affected.

Persistent chronic mild under-nutrition has been reported to characterise the anthropometric status of children in South Africa (Swart *et al*., 2008). The SAVACG survey in 1994 suggested that 9% of the children were underweight and 23% were stunted (SAVACG, 1995) at the time. More recent data (NFCS-FB, 2005) suggest that the percentage of underweight children in South Africa had remained relatively unchanged at 9% and that the percentage of stunted children had improved from 23% to 18% (Kruger *et al.,* 2007).

In the past, attention was focused on the negative consequences of inadequate intake of protein and energy, but attention is now shifting more towards the important role that micronutrient deficiencies could play in the cognitive development of children (Black, 2003). Micronutrients such as iron, zinc, and iodine (Black, 2003; Eilander *et al.,* 2010; Hubbs-Tait *et al*., 2005) have all shown to play a role in cognitive function.

2.2.1 Zinc and cognitive function

The trace element, zinc, plays both a functional and a structural role in the brain (Black, 1998). Children may be particularly vulnerable to zinc deficiency during periods of rapid growth, such as infancy and adolescence (Black, 1998). In developing countries, diets tend to be low in animal products and high in phytates, which can bind zinc and iron, increasing the risk of zinc/iron deficiency in these children (Bhan *et al.*, 2001).

Animal studies have suggested that cognitive development may be influenced through associations between zinc and decreased activity and possibly also emotional behaviour, but human data remain limited (Bhatnagar & Taneja, 2001). Some zinc supplementation studies in humans have reported no difference in mental concentration, short-term memory (Cavan *et al*., 1993) or in attention span scores (Gibson *et al*., 1989) for children between the ages of five and eight years. However, when school-aged children between the ages of six and nine years from low-income families in China were supplemented with zinc, significant improvement in neuropsychological performance (Cognition-Psychomotor Assessment System-Revised, CPAS-R) was reported (Sandstead *et al.,* 1998). Tests conducted included visual-motor tracking, sustained attention, abstract reasoning, fine and gross motor skills and eye-hand coordination.

This may point towards a possible effect of zinc on cognitive function, which deserves some research attention.

It has been suggested that zinc deficiency could affect cognitive performance through changes in attention, activity and neuropsychological functioning (Black, 1998). However, more research is needed in order to understand the biological mechanisms, critical periods, as well as the threshold of severity of zinc deprivation in cognitive development (Bhatnagar & Taneja, 2001).

2.2.2 Iodine and cognitive function

Iodine plays an important role in the production of thyroid hormones. These hormones are essential for physiological regulation of normal brain development (Bernal & Nunez, 1995). When iodine deficiency occurs *in utero*, foetal hypothyroidism is caused and irreversible neurological and cognitive deficits can manifest as cretinism (Black, 2003). Cretinism is the most serious iodine-deficiency disorder, characterised by serious mental impairment and physical abnormalities (Gordon *et al*., 2009).

Of much greater public health concern than cretinism, regarding iodine deficiency, are the subtle degrees of brain damage which may result in reduced cognitive capacity, affecting larger groups of populations (WHO, 2007). Some studies have reported the *in utero* effect of iodine deficiency on motor and cognitive performance (Cao *et al*., 1994; Pharoah & Connolly, 1987), but the postnatal effect seems less clear (Zimmermann *et al.*, 2006).

Zimmerman *et al.* (2006) reported that information processing, fine motor skills, and visual problem solving in moderately iodine-deficient schoolchildren improved with iodine repletion. Gordon *et al.* (2009) has published results indicating that iodine supplementation may result in improved perceptual reasoning in mildly iodine-deficient children and that these results suggest that mild iodine deficiency may prevent children from attaining their full intellectual potential. As a result of iodine deficiency, the mental ability of apparently normal children, as well as of adults, has been reported to be reduced in comparison with what it could have been if these children and adults had not previously been iodine deficient (WHO, 2007).

2.2.3 Micronutrient interactions with cognitive function

As indicated above, studies have investigated particular micronutrients and cognitive function. However, nutrients do not function in isolation, and a diet that is deficient in one micronutrient is most likely to be deficient in others too (Benton, 2008). Failure to find positive results regarding the supplementation of a single micronutrient could be due to this state of multi-micronutrient deficiency and, therefore, it is important to conduct studies providing multi-micronutrient supplements.

In 2003 researchers conducted a multiple-micronutrient-fortified fruit powder beverage intervention involving 808 Filipino schoolchildren in grades one to six (Solon *et al.,* 2003). At baseline, 52% of the children were anaemic (Hb < 12 g/dl) with five percent of children being severely anaemic (Hb > 8 to <10 g/dl). After 16 weeks consumption of the micronutrient-fortified beverage, significant effects on nonverbal mental ability scores were reported.

Vazir *et al.* (2006) supplemented children with a micronutrient-fortified beverage for a period of 14 months. Attention-concentration was significantly improved, but intelligence quotient (IQ), memory or school achievement was not influenced even though there was a significant improvement in the micronutrient status of the micronutrient-supplemented group. The children in the study had above-average IQ at baseline, and came from middle-income groups (Vazir *et al.,* 2006). It is therefore possible that the lack of effect on IQ may have been because of the already relatively high IQ at baseline. In 2010, another study was conducted where a multivitamin was administered for a period of four months to schoolchildren in households with median incomes (Perlman *et al.,* 2010). As with the study of Vazir *et al.* (2006), school performance did not improve significantly when compared with the placebo group. Unfortunately, baseline blood values of vitamins and minerals were not measured as in the study of Solon *et al.* (2003) and it is, therefore, not known if the children were micronutrient deficient. It can be argued that the above results could have been different if the same intervention had been conducted in a population group characterised by low micronutrient levels, such as is known to be the case with South African children.

2.3 Different levels of iron status

Around 80% of human body iron is stored in haemoglobin (Hb), a protein in red blood cells. Iron is stored as serum ferritin (SF), a protein found in the blood, liver, bone marrow and spleen. Changes in the levels of Hb and SF can indicate progression of iron deficiency (Hubbs-Tait *et al*., 2005). As the different body compartments become iron-depleted and iron deficiency develops, different measurements of iron status are used and no single biochemical index can measure all stages. In the first stage, adequate iron remains to meet the needs of red cell production, but iron stores are depleted (MacPhail, 2007:132). At this stage Hb would be normal but SF would be low. In the second stage, the amount of circulating iron drops when the iron stores are exhausted but Hb remains normal. Red cell production is now compromised (iron-deficient erythropoiesis) and is reflected by high serum transferrin receptors (STR), followed by the final stage, where anaemia develops. Anaemia is defined as low Hb concentration in the blood, based on an age-appropriate reference value, and iron deficiency is reduced SF to a certain value (MacPhail, 2007:135), as shown in Table 2.2.

Table 2.2: Selected biochemical iron status indicators and the levels below which poor iron status is present

Age group	Hb (g/dl) (Anaemia)	Haematocrit (mmol/l) (Anaemia)	SF (µg/l) (Depleted iron stores)
Children 6 months to 59 months	11	6.83	12*
Children 5 – 11 years	11.5	7.13	15
Children 12 – 14 years	12	7.45	15

Adapted from WHO (2001)

Hb, Haemoglobin; RBC, Red blood cell; SF, Serum ferritin

*For children with infection the value indicating depleted iron stores for serum ferritin is 30 µg/l

Iron deficiency in the developed world has been described as being largely a single nutritional problem that results from insufficient dietary intake of iron (Eden, 2005; Olivares *et al.,* 1999). In developing countries, causes of iron deficiency include diets high in phytate, chronic anaemia, protein and energy malnutrition, vitamin A and folate deficiency and infection. The main cause, however, remains low intake of bio-available iron. Under-nutrition, specifically reduced iron intake, leads to anaemia. Generally, the time of highest risk for iron deficiency has been

identified as occurring during periods of rapid growth and high nutrient demand (Black, 2003), such as in infancy, or among young children and women of reproductive age.

The consequences of iron deficiency on cognition have been most strongly demonstrated among children with the highest prevalence of iron deficiency (Halterman *et al*., 2001). The extent and timing of iron deficiency may influence brain function (Beard & Connor, 2003). Thus, both duration of iron deficiency and the time at which anaemia is present in a child's lifespan could influence the extent to which brain function may be affected. Both iron deficiency anaemia (Leslie & Jamison, 1990) and iron deficiency without reaching Hb levels to be classified as anaemia have been associated with increased risk of poor cognitive development (Grantham-McGregor & Ani, 2001). Children that are anaemic usually have poorer cognition than those without anaemia (Grantham-McGregor & Ani, 2001).

It seems that iron deficiency anaemia (IDA) may not act as a threshold at which cognition is affected. Bruner *et al.* (1996) found that in non-anaemic adolescent girls between the ages of 13 to 18 years iron supplementation improved certain aspects of cognitive function, such as verbal learning and memory. Similar results were published in the United States, where the National Health and Nutritional Examination Survey III (NHANES) investigated the relationship between iron deficiency and cognitive test scores (Halterman *et al.,* 2001). The study included 5398 children aged 6-16 years. Children with iron deficiency (classified as two of the three values of ZnPP, STR, SF to be abnormal for age and gender), with or without anaemia (classified by Hb level), had lower average scores for standardised mathematics tests (Wechsler Intelligence Scale for Children - Revised and the Wide Range Achievement Test - Revised) than those with normal iron status. Therefore, it seems that levels of iron status do not need to decrease to such a degree as to be classified as IDA to influence the cognitive function of a child. The influence of iron status on cognition function will be covered in more detail in section 2.5.

2.4 The role of iron in the nervous system

The exact mechanism by which a shortage of iron impairs cognitive function is not yet fully understood (Eden, 2005). It is, however, important to note that the number of studies on how iron affects brain function is limited, and that these have been conducted on animals. The areas that have been studied can be divided into the following three functional groups: firstly, oligodendrocyte metabolism and myelination; secondly, monoamine metabolism; and thirdly, gamma-aminobutyric acid (GABA) metabolism (Beard & Connor, 2003).

Oligodendrocytes are neuroglial cells with dendritic projections that coil around axons of neurons (MOSBY, 2006:1326) and are responsible for the production of myelin sheaths of the neurones of the central nervous system (OMD, 1998:459). Decreased availability of iron to oligodendrocytes could lead to decreased amounts of iron in the composition of myelin sheaths (Morley *et al.,* 1999). With iron deficiency severe enough to be classified as IDA, the neuron myelination and neurotransmitter synthesis may be influenced (Petranovic *et al*., 2008). Iron also acts as a cofactor for enzymes involved in neurotransmitter synthesis and the catabolism of neurotransmitters (Petranovic *et al*., 2008). Through the influence on nerve myelination and neurotransmitter synthesis, nerve impulse conduction may also be influenced. This may in turn influence the processing of information, affecting cognitive function.

It has also been suggested that the role of iron on the neurotransmitter systems could affect behaviour through the effect on the metabolism of the monoamine transmitter dopamine (Black, 2003). The rate of dopamine clearance from the interstitial space highly influences processing of environmental information (Beard & Connor, 2003). Therefore, alterations in dopamine metabolism could influence attention, perception, memory, motivation and motor control (Beard & Connor, 2003). Each of these factors can affect cognitive function in some way.

2.5 The influence of iron on cognitive function

Although numerous studies have been conducted to investigate the relationship between iron status and cognition, it seems to remain a somewhat controversial topic. The neurological manifestation of iron deficiency, namely impaired cognitive function, is often unrecognised and overlooked (Eden, 2005).

In a large, well-designed prospective follow-up study by Walter *et al.* (1989) (n=196 infants), iron supplementation was administered over a period of 10 days or three months to subjects where each intervention group had a corresponding placebo group. Mean Hb levels at baseline were 10g/dl for anaemic infants, indicating that anaemia was mild. At baseline, anaemic infants had significantly lower mental and psychomotor developmental scores than non-anaemic infants between 9 and 15 months of age. The intervention results showed no difference in the effect of oral supplementation of iron after 10 days compared with three months of supplementation (Walter *et al*., I989). This led the authors to suggest that cognitive impairment caused by iron deficiency could be long-standing and perhaps irreversible. Although a study duration of 12 weeks has been shown to be sufficient to alter iron status in order to increase oxygen supply to the tissues (Falkingham *et al.,* 2010), much longer intervention periods are needed for outcomes such as scholastic achievement. Researchers have been urged to initiate well-powered, blinded studies with study durations of at least one year among children (Falkingham *et al.,* 2010) for developmental effects takes longer to manifest.

Pollit *et al.* (1986) reviewed the main findings of published studies after 1976 up to 1986. The studies reviewed were conducted to evaluate the possible effects of iron deficiency on cognitive function in infants and preschool children. They found that iron-deficient infants and children from populations with a high prevalence of protein-energy malnutrition may be less likely to improve their cognitive performance after iron repletion therapy. Results also suggested that preschool children with IDA may have a higher likelihood of deficits in attention and higher order cognitive functions such as conceptual learning (Pollitt *et al*., 1986).

In an observational study by Hurtado *et al.* (1999), birth records, school records and the Woman, Infant and Child Programme records of 5411 subjects were evaluated at the age of 10 years. Results suggest that an increased probability for mild to moderate mental retardation is associated with anaemia in children, independent of maternal education, gender, birth weight, race ethnicity and the mother's age.

Based on the information presented above, the types of effects that iron may have on brain function may, therefore, influence cognitive function in schoolchildren, which could in turn, affect long-term educational outcomes. Furthermore, malnutrition has been associated with reduced cognitive function and it is, therefore, not surprising that many countries have introduced school feeding as a method of impacting the nutritional status of school children. South Africa is no exception and in 1994 the South African government introduced the Primary School Nutrition Programme (PSNP) (PSC, 2008:viii). The programme was later renamed the National School Nutritional Programme (NSNP).

2.6 Micronutrients and school feeding in South-Africa

In South Africa, poverty has been reported to be a possible major factor in food deprivation among a large number of young children who, therefore, are not able to participate fully in their own educational development (Wildeman & Mbebetho, 2005). Hunger in children may thus have significant implications for cognitive performance, and remains a problem in both developed and developing nations (Fanjiang & Kleinman, 2007), but more so in developing countries.

Owing to the high prevalence of poverty across South Africa, the government established the PSNP in schools in 1994 (PSC, 2008:x). It was recently reported that the programme provided one daily meal to more than six million learners (Department of Basic Education, 2010:4). The aim of the programme is to enhance the educational experience of needy primary school learners through promoting punctual school attendance, alleviating short-term hunger, improving concentration and contributing to general health (Department of Basic Education, 2008:1).

The contribution of energy by the school feeding programme to the children's recommended daily allowance (RDA) has been changed over time. To reduce costs, in 1995 reductions were made to the national contribution for the RDA for energy (Table 2.3) (CHU & HST, 1997:53). The percentage of RDA provided for energy was reduced from 30% to 25% for children of 7 to 10 years of age and to 20% for children of 11 to 14 years of age (CHU & HST, 1997:53). In 1997, an evaluation of the NSNP reported that out of 33 meals analysed, only six managed to provide more than 25% of the RDA for energy for children of 7 to 10 years of age (CHU & HST,

1997:53). This would seem to indicate that the programme may be struggling to adhere to the prescribed RDA contribution for energy.

A report on the Evaluation of the NSNP done in March 2008 included only two of the nine provinces (PSC, 2008). In this report, increased enrolment of learners at schools, increased school attendance and improved participation by the learners in the classrooms were reported as possible outcomes of the impact of the programme (PSC, 2008:x). It seems that even though there were difficulties in reaching the goal of RDA for energy, some benefits at school level were nevertheless observed. The reduced energy content of the meals could, however, imply less impact on the child's nutritional needs and there could even be a minimum point at which no further impact on the improvement of school performance may be observed (CHU & HST, 1997:53).

Table 2.3 Change in specifications for the school feeding programme

Original objective 1994	1995
• To meet 30% of the daily energy needs of primary school children	• To provide not less that 25% of the RDA for 7- to 10-year-old children • To provide not less than 20% of the RDA for 11- to 14-year-old children

RDA, recommended daily allowance — Adapted from CHU & HST (1997)

Useful contributions have been made by the NSNP regarding its contribution to the energy intake of South African children, in so far as this energy is additional to what they would normally consume. Schoolchildren receive a certain quantity of micronutrients as a result of fortified maize meal and flour (Foodstuffs, Cosmetics and Disinfectants Act, no 54/1972) (FCDA, 54/1972). Even though the programme does not target improvement of micronutrient intake as a goal, the NSNP would be indirectly addressing micronutrient intake through the fortification legislation. Unfortunately, the contribution made in terms of micronutrient fortification has not been investigated. Micronutrient fortification includes Vitamin A, thiamin, riboflavin, niacin, folic acid, pyridoxine, zinc and iron (FCDA, 54/1972). Wheat foodstuffs are fortified with 43mg/kg iron, and maize foodstuffs (maize products that are used to produce maize pap) with 37mg/kg. The iron used for fortification is electrolytic iron, which, unfortunately has low bioavailability in a

maize diet. Because of the fact that the iron status of South African children has been reported to be problematic and because school feeding does not directly target micronutrient intake, one cannot be sure of the effect that fortification may have on the iron status of children receiving the school meals. It would, therefore, be useful to investigate the possible influence of the iron status of South African primary school children on cognitive function. The aim of this mini-dissertation is, therefore, to determine whether there is an association between iron status, anthropometric indicators of nutritional status and cognitive performance in black South African children. Chapter 3 includes an article based on the research conducted to address this aim.

CHAPTER 3:

ARTICLE: Iron status, anthropometric status and cognitive performance of black African school children aged 6-11 years in the Klerksdorp area

Iron status, anthropometric status and cognitive performance of black African school children aged 6-11 years in the Klerksdorp area

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Keywords: cognitive performance, black African children, anthropometry, iron status

Abstract

Design: Cross-sectional study of the Beverage Fortified with Micronutrient (BeForMi) study population at baseline.

Setting: Three public primary schools in a peri-urban settlement of North-West Province, South Africa.

Subjects: Children aged 6 to 11 years (n=407), with written parental/guardian consent.

Results: Wasting (BMI-for-age) (BAZ ≤ -2) (8.4%), stunting (Height-for-age (HAZ) ≤ -2) (12.8%) and underweight (Weight-for-age (WAZ) ≤ -2) 14%) were present. Seven percent were anaemic and 15% had low body iron stores (Serum Ferritin (SF) < 12µg/L). Less than 3% had iron deficiency anaemia (IDA). No difference in SF was found between children with and without elevated C-reactive protein (CRP). Positive correlations were observed between SF and WAZ ($r = 0.10$, $p = 0.047$), Haemoglobin (Hb) and HAZ ($r = 0.13$, $p = 0.007$) and Hb and WAZ ($r = 0.13$, $p = 0.009$). All z-scores were positively correlated ($p < 0.05$) with cognitive global scales of the Kaufmann Assessment Battery for Children, Second Edition (KABC-II). Negative correlations were observed between the KABC-II sub-tests Triangles and Rover (sub-tests of simultaneous processing) and Hb ($r = -0.13$, $p = 0.008$) and SF ($r = -0.10$, $p = 0.04$). Children without IDA had significantly higher HAZ. There were no differences in KABC-II global scales for children with and without IDA.

Conclusions: In this study population, results suggest that under-nutrition was associated both with poor iron status and lower cognitive scores. The negative correlations observed between current iron status and selected cognitive sub-test scores, though with small effect sizes, warrant further research.

Introduction

Millions of children in developing countries, including South Africa, fail to reach full cognitive developmental potential because of exposure to poverty, malnutrition, poor health and insufficient homecare lacking in stimulation[1]. Stunting, inadequate cognitive stimulation and iron deficiency anaemia (IDA) have been named as key risk factors for compromised development in children under five years old[2].

The poor iron status of children worldwide has been of concern for decades. In South Africa, the latest National Food Consumption Survey-Fortification Baseline (NFCS-FB-2005) reported that six percent of children aged between one and nine years were iron deficient and 28% were anaemic[3]. The association between iron deficiency and cognitive function in early childhood is well documented[4,5], but data for primary school children are lacking. Although the National Food Fortification Programme (NFFP), implemented in 2003[6], was aimed at improving micronutrient status, results from two national surveys (South African Vitamin A Consultative Group (SAVACG-1994 and NFCS-FB-2005) suggested that iron status had deteriorated in children from one to five years of age[3].

Chronic and acute malnutrition contributes to poor iron and nutritional status among South African children. In 2005, stunting and wasting among South African children from one to nine years old were reported to be 18% and 4.5% respectively, with 9.3% of children being underweight[7]. Malnutrition, especially chronic under-nutrition by age two, has been associated with poor cognitive function in late childhood[8,9].

The aim of this study was, therefore, to investigate the relationship between iron status indicators, anthropometric nutritional status and cognitive performance of a sample of primary school children aged 6-11 years.

Experimental methods:

Study design

This was a cross-sectional study of the Beverage Fortified with Micronutrient (BeForMi) study population. The BeForMi study was a double-blind placebo-controlled intervention study involving primary school children in the North-West Province, South Africa. This study investigated associations between iron status indicators, anthropometric nutritional status and cognitive performance of the BeForMi study population at baseline.

Setting and subjects

Three schools that had the greatest potential to benefit from micronutrient supplementation were selected by the Department of Education from a peri-urban settlement. Two of the schools used Setswana and one isiXhosa as the primary medium of education. A power calculation was made for 80% power and a 0.05 level of significance for detection of a medium effect size (0.4) on cognitive scores for the parent BeForMi study. The total BeForMi study population of 414 was used as a convenience study sample for the present cross-sectional study (Figure 3.1).

Inclusion and exclusion criteria

The children included had to attend one of the three selected schools, be 6-10 years old by January 2010, have no apparent health condition that could influence the practicality of cognitive testing, and be taking no medication or supplements that could affect nutritional status (Figure 3.1). Signed informed consent of a parent or guardian was obtained for each child. The child had to be willing to have a blood sample taken. Children were excluded if the parent or guardian, because of disability, was not able to understand what the project required. For children with parental consent, firstly, those with the highest transferrin receptor (STR) and thereafter, the 414 with the highest Zinc Protoporphyrin (ZnPP) values, were selected (Figure 3.1).

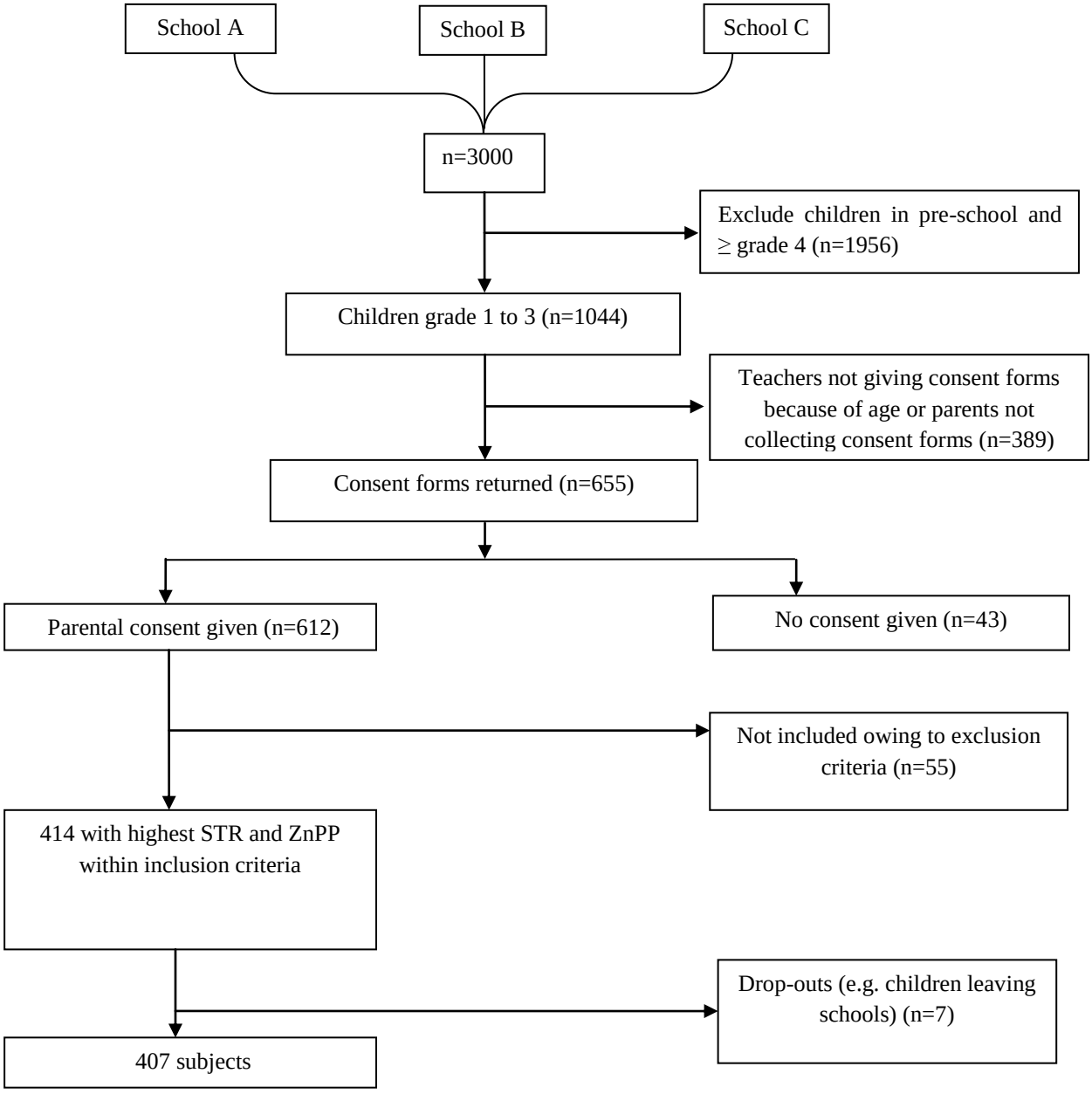

Figure 3.1 Study design.

n, sample size; STR, Serum Transferrin Receptor; ZnPP, Zinc Protoporphyrin

Owing to the selection criteria, the group selected had to be of low iron status and was thus not representative of the population. However, the study is relevant as it would provide a snapshot of the situation for a population sample at risk of low iron status.

Ethical approval

The Research and Ethics Committee of the North-West University (NWU) granted ethical approval for the BeForMi study (reference number NWU-00065-09-A1), which also covered this study. The Department of Education granted permission for the study and identified the three schools. The study was registered with the North-West Department of Health (registration number NWEP 04/2010).

Consent forms compiled for the BeForMi study were translated from English to Setswana, IsiXhosa and Afrikaans, the languages mostly spoken in the catchment area of the schools. Parents collected forms in their preferred language. Meetings with parents were held to provide full details of the project plans. At these meetings, where parents could ask questions freely, letters and consent forms were distributed, but if parents/guardians could not attend, children were given forms to take home. If parents/caregivers requested additional explanation, the school assistants visited the homes to answer any questions. The project staff collected the signed and unsigned forms from the teachers to whom the children had returned them. A child could withdraw from the study freely or be withdrawn by parents/guardians at any time without question. All of the information gathered was treated as strictly confidential.

Data collection

Recruitment and training of cognitive assessors and school assistants

School assistants and cognitive assessors were recruited and trained by the BeForMi study team. School assistants and assessors needed to speak Setswana and/or IsiXhosa fluently with a minimum qualification of a grade 12 certificate and have been trained to carry out the required tasks. School assistants administered the socio-demographic questionnaire. The task of the assessors included the cognitive assessment of children in the study.

School Assistants

Seventeen potential assistants were interviewed, sixteen of whom were intensively trained and tested on their ability to perform the tasks required by the study, and, based on these tests, twelve assistants were selected for the project.

Cognitive assessors

Sixteen applicants were interviewed and ten shortlisted for training. Five were recruited based on tests conducted during the training process. Three assessors from a previous study, together with the five newly recruited applicants, underwent further training before the assessment was done. Inter-tester reliability was determined through a test-retest process involving children in two schools not part of the study. In addition, adapted versions of the Cronbach alpha equation according to Foxcroft and Roodt[10] were used to determine intra-tester reliability coefficients based on the first eight children assessed by each assessor.

Outcomes measured

Anthropometry

The International Standards for Anthropometric Assessment of the International Society for the Advancement of Kinanthropometry (ISAK)[11] were followed for measurements.

Weight (kg) was measured with children in minimum school clothing and no shoes, and recorded to the nearest 100 g using a SECA Robusta 813, digital scale (Hamburg, Germany). Height (cm) was measured to 0.1 cm with a calibrated stadiometer with the child standing without shoes and upright with the head in the Frankfort plane.

Weight-for-age (WAZ), BMI-for-age (BAZ) and height-for-age z-scores (HAZ) were computed using 2006 World Health Organization (WHO) standards and the specified WHO Anthropometry software, version 3.0.1[12]. The validity of the WAZ decreases in children older than ten and therefore only children aged ten years and younger were included (n=391) for WAZ analysis. Children up to 11 years old were included in HAZ and BAZ analysis.

Blood sample collection

A total of 10 ml of blood, which included a venous EDTA blood sample of 4 ml and a 6 ml sample in a tube free of trace elements, was collected from each child. Blood was transported on ice to the laboratory. Haemoglobin (Hb) was measured using an AcT 5Diff Cap Pierce Hematology Analyzer (Beckam Coulter, Miami, Florida, USA). Enzyme-linked immunosorbent assays were used to measure STR and serum ferritin (SF) (Ramco Laboratories Inc.). ZnPP was measured with a hematofluorometer (Aviv Biomedical, Lakewood, NJ, USA). Serum C-reactive protein (CRP) was measured through immunoturbidimetric test (Human Biochemical and Diagnostic Laboratories, South Africa). Hb and ZnPP were determined from whole blood and STR, SF and CRP from serum.

Diagnostic threshold values were chosen with reference to WHO recommendations and the test kit recommendations as follows: SF (< 12-15 µg/L) (3rd International Standard, 1996, 94/572) and CRP (< 5 mg/L)[13], Hb (< 11.5 g/dL)[3], STR (2.9-8.3 mg/L) (Ramco Laboratories Inc.) and ZnPP (≤ 70 µmol/mol heme) (Aviv Biomedical, Lakewood, NJ, USA).

Cognitive assessment

The cognitive assessment was overseen by a registered psychologist. For the purpose of this study, a selected number of cognitive sub-tests was chosen from the Kaufmann Assessment Battery for Children (KABC-II)[14] (Table 3.1). The raw scores obtained were used to generate sub-test scaled scores and global scores as an indication of cognitive performance using the KABC-II Assist software[14]. All of the sub-tests were used to compute the global scale score Mental Processing Index (MPI), while only sub-tests not affected by verbal ability were used for the Non-Verbal Index (NVI) (Table 3.1). Both of the global scales correlate well with other Intelligence Quotient measures[15].

Cognitive testing was done during morning school hours on the school premises. The instructions to the children were translated and given in a standardised manner to each child in either Setswana or isiXhosa, according to the language used as medium of education for the particular school. The scores were used to investigate correlations between iron status indicators and anthropometric Z-scores and were not used for psychological diagnosis or interpretation. Although the KABC-II has not been standardised for South African children, it has been demonstrated not to be highly susceptible to cultural bias, making it suitable for use in this population setup[15].

Socio-demographic data

An existing socio-demographic questionnaire was adapted for use in this study. It was administered by the trained school assistants who interviewed parents/caregivers in their language of choice to collect the socio-demographic information of the children.

Table 3.1 KABC-II scales and sub-tests used

KABC-II scales	Sub-tests
Planning Ability: High-level skills, required for efficient problem solving.	Story completion*
Simultaneous Processing: Visual spatial processing and conceptualisation in order to arrive at a solution as a whole.	Triangles* Rover
Sequential Processing: Reflects on short-term memory in terms of ability to arrange information sequentially and/or serially to solve a given problem	Hand Movements* Word Order Number Recall
Learning Ability: Reflects on the integration of attention-concentration processes, ability to code and store new information based on audio and visual stimuli and retention of new information	Atlantis

Adapted from Kaufman *et al*. (2005)[14]

* Used to compute Non-Verbal Index scaled score
KABC, Kaufmann Assessment Battery for Children

Statistical analysis

Statistical analysis was done using the computer software package SPSS (SPSS Inc, 2009), Statistica (StatSoft Inc, 2011) and SAS (SAS Institute Inc, 2003) in collaboration with the NWU Statistical Consultation Services. For statistical significance a p-value ≤ 0.05 was used. Continuous data were reported as mean (SD) and categorical data were reported as median (25^{th} and 75^{th} percentiles). Cognitive test were seen as categorical for although scores are ordinal, it's not strongly continues.

Spearman's rank order correlation coefficients were determined between iron status indicators and anthropometric z-scores, anthropometric z-scores and cognitive scores, and iron status indicators and cognitive scores. Independent T-tests for equality of means were conducted to compare anthropometric z-scores and cognitive global scales (NVI & MPI) between children with IDA and those without. T-tests were also conducted to compare SF values for children with and without elevated CRP levels. Because SF is an acute-phase protein, the correlation between CRP and SF was determined.

Effect sizes for significant correlations were regarded as small, medium and large for r-value cut-offs of 0.1, 0.3 and 0.5 respectively. For comparison between means the effect size cut-offs for Cohen's d-value were 0.2, 0.5 and 0.8 for small, medium and large respectively[16]. Effect size is a useful indicator of the practical importance of research results[17].

Odds ratios with IDA as the dependent variable were determined for WAZ, HAZ and BAZ, as well as the head of the household's education level and the number of people in the household, as independent variables to determine the likelihood of these factors being associated with IDA. Because of the small number of children with IDA, the same independent variables were also used, with the dependent variable being the likelihood of the children falling into the upper quartile or lower quartile, when children were divided in quartiles according to Hb levels. Odds ratios for cognitive scores were not determined because the KABC-II scores were not intended for diagnostic purposes.

Results:

Socio-demographic data

The number of children included in the study was 407 (48.2% girls). Thirty eight percent of the children lived in households (HH) of 4 - 6 persons, 19.2% in HH of < 4 persons and 32.7% in HH of ≥ 7 persons. More than half (56.8 %) of the children were from HH where the head of the HH earned less than R 2000 rand (300 USD) per month. Although 25.6% and 37.6% of the HH heads had primary and secondary education respectively, almost 20% had no schooling.

Anthropometric data

Fourteen percent of the children were underweight (WAZ ≤ -2 SDs) and of these only 2.5% were severely underweight (WAZ ≤ -3 SDs). Approximately 13% were stunted (HAZ ≤ -2 SDs) and 8.4% wasted (BAZ ≤ 2 -SDs). Only 1% and 1.5% of the children had WAZ and BAZ ≥2 respectively, indicating low prevalence of overweight.

Iron status indicators and CRP levels

Just over 7% of the children were anaemic (Hb < 11.5 g/dL), 4.1% moderately anaemic (Hb = 7 - 10 g/dL) and none severely anaemic (Hb < 7 g/dL). The mean Hb and SF values were 12.65 g/dL and 30.56 µg/L respectively (Table 3.2). More than 15% had low body iron stores (SF < 12µg/L). Twenty four percent and 7.6% of the children were above ZnPP and STR threshold values; both of these iron indicators serve as sensitive measures of iron-deficient erythropoiesis. Of the total study population, 2.7% (n = 11) had IDA (Hb < 11.5 g/dL and SF < 12 µg/L) and 13% were iron depleted (Hb ≥ 11.5 g/dL and SF <12 µg/L, cut-off values as were used in the previous NFCS-1994 & NFCS-FB-2005[3]).

Twenty six percent of children had serum CRP above the threshold value (CRP < 5 mg/L). There were no significant correlations between CRP and Hb, or STR or ZnPP. A significant correlation was observed between CRP and SF ($r = 0.17$, $p = 0.01$) but no difference ($p < 0.05$) in SF of children with and without elevated CRP ($d = 0.14$, $p = 0.19$) and therefore CRP was not corrected for subsequent analysis.

Table 3.2 Descriptive statistics for iron status indicators, anthropometric z-scores, cognitive global scales and scaled sub-test scores*

Variables	n	Mean	SD	Missing data	Variables	n	Median	25th& 75th Percentiles	Missing data
Biochemical indicators					**KABC II Sub-test scaled scores**				
Serum Ferritin (µg/L)	400	30.56	22.44	7	Atlantis	405	4	(2, 5)	2
Haemoglobin (g/dL)	406	12.65	0.96	1	Number recall	406	6	(5, 8)	1
Zinc Protoporphyrin (µmol/mol heme)	407	62.08	29.43	0	Rover	406	7	(6, 9)	1
Serum Transferrin Receptor (mg/L)	407	6.14	1.66	0	Story completion	394	4	(2, 5)	13
C-Reactive Protein (mg/L)	407	4.50	4.93	0	Triangles	393	5	(4,7)	14
					Word order	406	6	(5, 7)	1
					Hand movements	405	6	(5, 8)	2
Global cognitive scales*					**Anthropometric z-scores**				
Mental Processing Index	405	66.65	7.83	2	HAZ	407	-0.95	(-1.47, -0.25)	0
Non Verbal Index	407	66.68	10.86	0	WAZ	391	-0.92	(-1.63, -0.14)	1
					BAZ	407	-0.56	(-1.24, 0.08)	0

n, sample size; SD, Standard deviation; KABC-II, Kaufman Assessment Battery for Children II; HAZ, height-for-age; WAZ, weight-for-age; BAZ, BMI-for-age.
*Categorical data are reported as median (25th and 75th percentiles) and continuous data are reported as mean (SD)

Correlations between iron status indicators and anthropometric z-scores

Significant correlations were observed between SF and WAZ (r = 0.1, p = 0.047), and between Hb and HAZ (r = 0.13, p = 0.007) and WAZ (r = 0.13, p = 0.009) (Table 3.3). Children without IDA had significantly higher HAZ (mean = -0.85 ± SD 1.01) than those with IDA (mean = 1.52 ± SD 0.99) (d = 0.66, p = 0.05) but no difference was found for WAZ and BAZ.

Table 3.3 Correlations between iron status indicators and anthropometric z-scores

		Z-scores		
Indicator		**Height-for-age**	**Weight-for-age**	**BMI-for-age**
Serum Ferritin	r	0.08	0.10*	0.05
	p	0.11	0.047	0.3
	n	400	384	400
Haemoglobin	r	0.13**	0.13**	0.04
	p	0.007	0.009	0.40
	n	406	390	406
Zinc	r	0.01	0.05	0.07
Protoporphyrin	p	0.89	0.34	0.15
	n	407	391	407
Serum	r	0.06	0.07	0.08
Transferrin	p	0.26	0.16	0.12
Receptor	n	407	391	407

BMI, Body mass index; r, correlation; p, p-value; n, sample size

* Correlation is significant at p< 0.05 level

** Correlation is significant at p< 0.01 level

Correlations between KABC-II scores and anthropometric z-scores

Cronbach alpha for inter-tester reliability for cognitive assessors was 0.76 (95% CI, 0.61; 0.87). The intra-tester reliabilities for the eight assessors ranged from 0.67 to 0.91 (average = 0.80).

There were significant correlations between some cognitive scores and z-scores, with the highest r-value observed being 0.24 for the Story Completion sub-test and WAZ (p = 0.0001) (Table 3.4). This correlation (r = 0.24) reflected close to a medium effect size. A similar trend was observed for the Triangles sub-test with HAZ, WAZ and BAZ, but with smaller effect sizes. MPI and NVI had significant positive correlations with all of the anthropometric z-scores (r < 0.2), but with small effect sizes.

Table 3.4 Correlations between KABC-II sub-test scaled scores, global scores and anthropometric z-scores

		KABC-II Sub-tests							KABC-II global scores	
		Planning ability	Simultaneous processing		Sequential processing			Learning ability		
Z-scores		**Story completion**	**Triangles**	**Rover**	**Word order**	**Hand movements**	**Number recall**	**Atlantis**	**MPI**	**NVI**
Height-for-age	n	394	393	406	406	405	406	405	405	407
	r	0.23**	0.11*	0.07	0.01	0.03	0.03	0.08	0.15**	0.15**
	p	0.0001	0.032	0.17	0.86	0.49	0.61	0.13	0.003	0.002
Weight-for-age	n	378	377	390	390	389	390	389	389	391
	r	0.24**	0.14**	0.05	-0.002	0.03	0.05	0.105*	0.16**	0.16**
	p	0.0001	0.008	0.29	0.98	0.54	0.30	0.038	0.002	0.001
BMI-for-age	n	394	393	406	406	405	406	405	405	407
	r	0.19**	0.11*	0.06	0.01	-0.01	0.05	0.06	0.12**	0.11*
	p	0.0001	0.034	0.24	0.93	0.89	0.32	0.20	0.017	0.025

KABC-II, Kaufmann Assessment Battery for Children, Second Edition; MPI, Mental processing index; NVI, Non-verbal index; n, sample size; r, correlation; p, p-value; BMI, Body Mass Index

* Correlation is significant at $p < 0.05$ level

** Correlation is significant at $p < 0.01$ level

Correlations between KABC II cognitive tests and iron status indicators

Negative correlations (small effect sizes) were observed for the Triangles sub-test with Hb ($p = 0.008$, $r = -0.13$) and SF with Rover ($p = 0.04$, $r = -0.1$), both sub-tests on simultaneous processing (Table 3.5).

Table 3.5: Correlations for KABC-II sub-test scaled scores and global scores with iron status indicators (n = 387-407)

Iron status indicators		**SF**		**Hb**		**ZnPP**		**STR**	
		r	p	r	p	r	p	r	p
KABC-II Sub-tests	**Story completion**	-0.08	0.13	-0.04	0.46	0.09	0.07	0.01	0.83
	Triangles	-0.03	0.53	-0.13**	0.008	-0.06	0.25	0.04	0.4
	Rover	-0.1*	0.04	-0.04	0.38	0.03	0.59	0.04	0.42
	Word order	-0.01	0.92	0.01	0.83	-0.04	0.48	-0.03	0.6
	Hand movements	-0.21	0.68	0.57	0.26	-0.03	0.59	0.57	0.25
	Number recall	-0.00	0.96	-0.06	0.26	0.03	0.57	0.05	0.31
	Atlantis	-0.01	0.85	-0.03	0.61	0.021	0.67	0.05	0.31
Global scales	**Model MPI**	-0.1	0.08	-0.1	0.11	0.3	0.55	0.06	0.22
	Model NVI	-0.1	0.07	-0.02	0.63	-0.01	0.9	0.05	0.33

SF: Ferritin; Hb, Haemoglobin, ZnPP, Zinc protoporphyrin; STR, Soluble serum transferrin receptor; KABC-II, Kaufmann Assessment Battery for Children, Second Edition; MPI, Mental processing index; NVI, Non-verbal index; n, sample size; r, correlation; p, p-value;.

* Significant at p< 0.05 level
**Significant at p< 0.01 level

Odds ratios for association between anthropometric z-scores, selected socio-demographic indicators and IDA

HAZ and WAZ were significantly associated with the likelihood of a child's being classified with IDA ($p = 0.02$ and $p = 0.04$ respectively) (Table 3.6). With every unit increase in HAZ, a child was 54% less likely to have IDA and with each unit increase in WAZ, a child was 46% less likely to have IDA.

Table3.6 Logistic Regression analysis predicting the Odds for a child having IDA[a] in association with anthropometric z-scores and selected socio-demographic factors

Variable	n	B	SE	Odds Ratio	95%CI	p-value
Height-for-age z-score	329	-0.77	0.33	0.46	(0.24, 0.88)	0.02*
Weight-for-age z-score	314	-0.62	0.30	0.54	(0.29, 0.96)	0.04*
BMI-for-age z-score	329	-0.25	0.29	0.78	(0.44, 1.37)	0.39
Number of persons per household	295	0.304	0.29	1.36	(0.76, 2.4)	0.3
Education level of the head of the household	345	-0.31	0.25	0.73	(2.23, 0.84)	0.21

BMI, Body Mass Index; CI, Confidence Interval

[a] Iron deficiency anaemia Hb<11.5 g/dL and Ferritin <12 ng/ml

* p-value is significant at the 0.05 level

Owing to the small number of children with IDA, the logistic regression was repeated with children divided into upper and lower quartiles according to Hb values. Results similar to those found with IDA were found with HAZ ($p = 0.036$) and WAZ ($p = 0.032$) being significant. In addition, the education level of the head of the household was also found to be a significant ($p = 0.036$) independent variable.

Discussion

The study demonstrated significant but small effect-size correlations for HAZ and WAZ with Hb. SF correlated significantly with WAZ. WAZ and HAZ also correlated positively with cognitive global scales and selected KABC-II sub-test scaled scores. Unexpected negative correlations were observed between some iron status indicators (Hb and SF) and selected cognitive sub-test scaled scores. While Zimmerman *et al.*[18] reported STR and ZPP as iron status indicators in African children, this study is, to our knowledge, the first in Africa that has included ZnPP and STR along with SF and Hb to investigate association with cognitive outcomes.

The prevalence of anaemia among children (7.1%) in our study group is of mild public health concern, based on WHO ratings[19]. This prevalence rate is less than the 2005 South African national anaemia prevalence of 27.9%[3], which included children from one to nine years of age. Based on the selection criteria of the BeForMi study, higher anaemia prevalence was anticipated. It is possible, although one cannot be certain, that the NFFP that was started in 2003 could have influenced the iron status of the children to this extent. Almost 16% of the children had low iron stores compared with the 14.5% of South African children reported to have low iron stores in 2005[3].

Fourteen percent of children in our study population were underweight, 8.4% were wasted and 13% stunted. Data from the NFCS-FB-2005 reported that 9.3% of children between one and nine years were underweight, 4.5% wasted and 18% stunted[7]. Unfortunately, comparisons with data from the SAVACG-1994, NFCS-1999 and NFCS-FB-2005 would not be truly reflective of the situation because the reference cut-off points of the National Centre for Health Statistics (NCHS) were used in all previous national surveys, while new WHO standards were used in the present study.

Intra-tester and inter-tester reliabilities can be important sources of variability in cognitive tests. The intra-tester reliability of the assessors was high indicating a high level of consistency in the assessment process by individual assessors. The inter-tester reliability was also high indicating lower variability contribution by individual assessors than by individuals being assessed. The

positive correlations observed for HAZ, WAZ and BAZ with story completion and the Triangles sub-test (reflecting planning ability and simultaneous processing, respectively) as well as global scales (MPI and NVI) were as expected. Kordas *et al.*[20] reported similar results in Mexican first-grade children. They observed correlations between stature and some cognitive outcomes based on the Wechsler Intelligence Scales for Children-Revised Mexican Version (WISC-RM). Although the study involved a different population and set of cognitive tests, these observations suggest positive associations between good nutritional status and cognitive function. Malnutrition may influence cognitive function through acute hunger and chronic under-nutrition. Wasting (acute malnutrition) may have an immediate effect on cognitive function through the child being more apathetic, exploring the environment less, being less active[9] and perhaps also by influencing concentration through hunger[21]. On the other hand, stunting (chronic malnutrition) may have a long-term influence by affecting cognitive development[22].

Certain areas in the brain are not yet fully developed at the age of two years, and brain development continues throughout childhood[23]. Myelination of frontal lobes has been reported to be a slow process, starting at six months but possibly continuing into adulthood, and therefore the possibility exists that poor nutrition may affect development of frontal lobe functions during childhood[23]. Stunting early in life has been strongly associated with poor cognitive function later in life[24]. In South Africa, higher prevalence levels of stunting have been reported for children between one and three years (23.4%) than between seven and nine years (12%) of age[7]. Some researchers have also suggested that stunting observed at primary school level could have originated from chronic malnutrition in early childhood years[25,26,27]. This view is indirectly supported by the positive correlations observed between HAZ and some cognitive scores in our study. Early under-nutrition may, therefore, contribute to underdevelopment of certain areas in the brain such as the frontal lobes, resulting in reduced cognitive ability. The frontal lobes are responsible for the execution of higher order functions[28], some of which are measured by the KABC-II tests used in this study.

The negative correlations observed between Triangles and Rover sub-test scores and Hb and SF respectively, though with small effect sizes, were unexpected. It would have been more logical to observe positive correlations since good iron status is expected to influence cognitive function

positively. In addition, no difference in cognitive scores of MPI and NVI were observed between children with and without IDA. This despite some researchers having reported that IDA may not necessarily have a threshold for affecting cognition[29,30]. It could be that the prevalence of IDA in our study population was too low to observe significant differences between the two groups. Furthermore, what could have contributed to these results was the small variation in iron status, with only 7% being anaemic and less than 3% having IDA, together with the specific selection of a study population with low iron status.

As for the negative correlations between Hb and SF with Triangles and Rover sub-test scores, Lozoff *et al.*[4] indicated the possibility that the timing of iron deficiency and aspects of iron delivery during early brain development could be more important than ultimately obtaining normal iron levels in the brain. The iron status observed in the present study may, therefore, not be indicative of the past iron status of these children, making the interpretation of the associations difficult. Low levels of iron in the brain could influence neurotransmitter synthesis – involving enzymes such as serotonin, nor-epinephrine and dopamine[31] – while impaired myelination during infancy could interrupt the laying down of cognitive fundamentals [4]. The interruption in this process could lead to children with iron deficiency being at different developmental levels from those who are not iron deficient. The children included in our study were born between 1999 and 2003. A large number was therefore born prior to the NFFP initiated in 2003[6], meaning that there is a possibility that some children had deficient iron intakes and poor iron status at a time critical for their brain development. However, long-term negative cognitive development effects can not merely be contributed by poor iron status alone. The negative correlations may also be due in part to the influence of other environmental factors that play a role in cognition. It is possible that factors such as income, education level of the mother, the number of persons in the household and the level of stimulation that the child was exposed to may have played such a role as confounders as to lead to the unexpected negative correlations observed.

Significant positive correlations, but with small effect sizes, were observed in our study population between WAZ, HAZ and Hb. The NFCS-FB-2005 reported results similar to ours, showing significant correlations with Hb for WAZ and HAZ, with small effect sizes ($r < 0.1$). In

this study, the likelihood of a child being iron deficient was significantly associated with increased HAZ and WAZ. Sichieri *et al.*[32] reported significant positive correlations between Hb and anthropometric indicators (r-value ranging from 0.19-0.33) from a study involving rural Brazilian school children aged six to twelve years with high prevalence of anaemia (25.7%) and stunting (53%). In Croatian third-grade children, non-anaemic (Hb > 12mg/L) children appeared to have better nutritional status than mildly anaemic children, though significant differences were only reached for boys[33]. Both the above studies had higher anaemia rates (25.7% and 39.5-46.4% respectively) and smaller study samples (n=138 and n=60 respectively). One would expect to see stronger correlations, such as were observed by Sichieri *et al.*[32] (r=0.33). Since our sample size was large enough compared with the above studies, it is possible that if a higher prevalence of anaemia had been found in our study, stronger correlations might have been observed.

Since this is a cross-sectional study, one limitation is that we cannot link any causal relationships to our observations. Furthermore, for intervention purposes, the study population was selected for poor iron status and is therefore not truly representative at population level. Nonetheless, valuable observations are that, despite prevalence of mild anaemia in children, positive correlations with Hb were observed for WAZ and HAZ, and between SF and WAZ. In addition, positive correlations were observed for WAZ and HAZ with cognitive global scales, suggesting that under-nutrition was associated with both poor iron status and lower cognitive scores in this study population. Since stunting may have occurred earlier in childhood, investigating relationships of cognitive function and iron status in different age groups over time would be useful to assess the possible benefits of targeted interventions in this population. The weak negative correlations with small ES between current iron status and selected cognitive scores warrant further scrutiny.

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CHAPTER 4: GENERAL SUMMARY, CONCLUSION AND RECOMMENDATIONS

4.1 INTRODUCTION

The aim of the study was to determine whether there were associations between iron status, anthropometric indicators of nutritional status and cognitive performance in black African children aged 6-11 years in the North-West province of South Africa. The purpose of this chapter is, firstly, to summarise the main findings and draw conclusions based on them with regard to the given hypotheses that:

- Anthropometrical status of the children is positively associated with cognitive performance.
- Iron status of the children is positively associated with cognitive performance.

Secondly, some recommendations for future studies will be provided. This can be done in more detail here outside the word limitation of Chapter 3.

4.2 MAIN FINDINGS AND CONCLUSION

We have observed mild anaemia prevalence in our study population, even though we were expecting higher prevalence of anaemia as a result of the selection process used by the parent intervention study. It is not clear if this may have been due in part to an influence of the NFFP on the iron status of children; to ascertain this would require further investigation.

Low anthropometric indicators of malnutrition (indicated by underweight, wasting and stunting) were associated with both poor iron status and lower cognitive scores in the study population. Contrary to our expectations, the current iron status of the children was negatively associated with cognitive performance with small effect sizes. Possible reasons for such negative correlations warrant further investigation in order to establish if this was just an anomaly of this particular population sample.

4.3 RECOMMENDATIONS

- In 2003, the NFFP was implemented and the NFCS-FB-2005 was conducted to provide fortification baseline data. Six years have passed since the NFCS-FB-2005, and the prevalence of anaemia in this study suggests there may be need for a follow-up national survey. Such a survey would help to determine whether the NFFP might have been a contributing factor to the reduced prevalence of anaemia that we have reported in our

study. Furthermore, since anthropometric reference values differed in these studies, new national data based on WHO reference standards are needed as a basis for comparison with smaller cross-sectional studies such as this one.

- Because under-nutrition might have originated in early childhood, studies that focus on the iron status, cognitive function and nutritional status of children at different ages over a period of time could be beneficial in shedding light on the possible usefulness of targeted interventions.

- As has been previously mentioned, no causal relationship can be inferred from the results of this study owing to its cross-sectional nature. While it is possible to determine stunting prevalence in a study population, it is difficult to determine the duration of under-nutrition as well as the timing of chronic malnourishment and iron deficiency that may have contributed to this. While a prospective study would be able to infer causality, investigating the effect of chronic malnutrition and iron deficiency in early childhood on later cognitive performance poses ethical limitations because, when poor iron status is identified in infants or young children, corrective measures need to follow immediately. While it is difficult to apply animal model studies directly to possible effects in humans, there are similarities in the metabolism of iron. It is therefore recommended that animal model studies should be done to find answers on the effects of earlier chronic malnutrition and poor iron status on cognitive development and cognitive performance later in life. This could shed some light on further intervention strategies and under which circumstances interventions may be useful.

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6. ADDENDA

ADDENDUM A: SOCIO-DEMOGRAPHIC QUESTIONNAIRE

BeForMi Socio-demographic questionnaire

(All information in this questionnaire is confidential)

a. Interviewer Name:_____ **b. Interview Date:** _____

c. Subject number _____ **d. Name of Child:** _____

e. School: _____ **f. Date of birth of child: Day …….. Month …….. Year ……**

g. Did your child attend creche or preschool		
Year	**How long? (give months)**	**Comment**
2008		
2007		
2006		
2005		
2004		
2003		
2002		
2001		
2000		

h. Gender	**1**	**2**
	Male	Female

i. Home language	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	Zulu	English	Sesotho	Setswana	Xhosa	Afrikaans	Other specify

j. Type of dwelling: (***You can tick more than one block if necessary***)	**1**	**2**	**3**	**4**	**5**
	Brick, Concrete	Traditional mud	Tin	Plank, Wood	Other, specify

k. Number of people living in the your household (*Tick one*)	**1**	**2**	**3**	**4**	**5**
	<4 persons	4-6 persons	7-8 persons	>8 persons	Don't know

l. The people in your household who work (*Tick one*)	**1**	**2**	**3**	**4**	**5**	**6** (specify)
	Mother/mother figure to child	father/father figure to child	grandmother	grandfather	sibling	Other funds

m. Where do you get drinking water most of the time (*Tick one*)	**1**	**2**	**3**	**4**	**5**
	Own Tap	Public Tap	River, Dam	Borehole, Well	Other: Specify

n. What type of toilet does your household have? (*Tick one*)	**1**		**2**	**3**	**4**	**5**
	Flush	Pit	Bucket, Pot	Ventilated Improved Pit latrine (VIP)		Other (Specify)

o. What fuel is used for cooking most of the time in your household? (*You can tick more than one*)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	Electric	Gas	Paraffin	Wood/ coal	Sun	Open Fire	Don't know

p. Do you have access to electricity inside your house?	**1**	**2**
	Yes	**No**

q. Does your household have a working:	**1**	**2**	**3**	**4**	**5**
1. Refrigerator / Freezer	Fridge	Freezer	Fridge/freezer combination	None	Don't know

2. Stove (*You can tick more than one*)	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	Coal	Paraffin	Gas	Electric	With oven	Without oven	None

3. Washing machine	**1**	**2**
	Yes	No

4. Microwave oven	**1**	**2**
	Yes	No

5. Television	**1**	**2**
	Yes	No

6. Radio	**1**	**2**
	Yes	No

r. **Household Composition (Defined as people who regularly eat together)**

Name	Age (yrs)	Gender 1= Female 2= Male	Relationship to child 1=mother 2=father 3=sibling 4=aunt 5=uncle 6=grandmother 7=grandfather 8=other	Currently Schooling? 1 = Yes 2 = No	Head of House-hold (Mark X)	Marital status 1= Single 2= Married 3= Divorced 4= Widowed	Current perceived health status 1= bedridden/ disabled, 2= ill, 3=average health 4= good health 5= excellent	Educational level 1=no schooling 2= preschool 3=primary 4=secondary 5=tertiary	Monthly income 1= < 500 2=500-1000 3=1000-2000 4= 2000-2500 5=2500-3000 6=30000-3500 7=3500-4000 8= >4000

Access to Food/Food Security:

s. Do you grow any food or vegetables for self consumption? Tick the relevant one

Food		Vegetables	
1. Yes	**2.** No	**3.** Yes	**4.** No

t. How many days in the last month has your family run out of food completely? (Record the actual number of day.) ____days (if none then record 0)

u. How many days in the last month has your family not had enough food to eat? (Record the actual number of day.) ____days (if none then record 0)

v. How many days in the last month did you reduce the number of meals in a day because there was not enough food? (Record the actual number of day.) ____days (if none then record 0)

Health Service Utilization

Select one by marking the box with X:

w. What services does this household mostly utilize for their health needs?					
1. Public Hospital	**2. Private Doctor**	**3. Traditional Healers**	**4. Others**	**5. Public Clinic**	**6. None**

ADDENDUM B: AUTHORS GUIDELINES

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References to material available on websites should include the full Internet address, and the date of the version cited. Thus:

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Mathematical modelling of nutritional processes. Papers in which mathematical modelling of nutritional processes forms the principal element will be considered for publication provided: (a) they are based on sound biological and mathematical principles; (b) they advance nutritional concepts or identify new avenues likely to lead to such advances; (c) assumptions used in their construction are fully described and supported by appropriate argument; (d) they are described in such a way that the nutritional purpose is clearly apparent; (e) the contribution of the model to the design of future experimentation is clearly defined.

Units. Results should be presented in metric units according to the International System of Units (see Quantities, Units, and Symbols (1971) London: The Royal Society, and Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences (1972) London: The Royal Society – as reproduced in *Proceedings of the Nutrition Society* (1972) **31**, 239–247). SI units should be used throughout the paper. The author will be asked to convert any values that are given in any other form. The only exception is where there is a unique way of expressing a particular variable that is in widespread use. Energy values must be given in Joules (MJ or kJ) using the conversion factor 1 kcal = 4·184 kJ. If required by the author, the value in kcal can be given afterwards in parentheses. Temperature is given in degrees Celsius (°C). Vitamins should be given as mg or µg, not as IU.

For substances of known molecular mass (Da) or relative molecular mass, e.g. glucose, urea, Ca, Na, Fe, K, P, values should be expressed as mol/l; for substances of indeterminate molecular mass (Da) or relative molecular mass, e.g. phospholipids, proteins, and for trace elements, e.g. Cu, Zn, then g/l should be used.

Time. The 24 h clock should be used, e.g. 15.00 hours.

Units are: year, month, week, d, h, min, s, kg, g, mg, µg, litre, ml, µl, fl. To avoid misunderstandings, the word litre should be used in full, except in terms like g/l. Radioactivity should be given in becquerels (Bq or GBq) not in Ci. 1 MBq = 27·03 µCi (1Bq = 1 disintegration/s).

Statistical treatment of results. Data from individual replicates should not be given for large experiments, but may be given for small studies. The methods of statistical analysis used should be described, and references to statistical analysis packages included in the text, thus: Statistical Analysis Systems statistical software package version 6.11 (SAS Institute, Cary, NC, USA). Information such as analysis of variance tables should be given in the paper only if they are relevant to the discussion. A statement of the number of replicates, their average value and some appropriate measure of variability is usually sufficient.

Comparisons between means can be made by using either confidence intervals (CI) or significance tests. The most appropriate of such measures is usually the standard error of a difference between means (SED), or the standard errors of the means (SE or SEM) when these vary between means. The standard deviation (SD) is more useful only when there is specific interest in the variability of individual values. The degrees of freedom (df) associated with SED, SEM or SD should also be stated. The number of decimal places quoted should be sufficient but not excessive. Note that pH is an exponential number, as are the log(10) values often quoted for microbial numbers. Statistics should be carried out on the scalar rather than the exponential values.

If comparisons between means are made using CI, the format for presentation is, e.g. 'difference between means 0·73 (95 % CI 0·314, 1·36) g'. If significance tests are used, a statement that the difference between the means for two groups of values is (or is not) statistically significant should include the level of significance attained, preferably as an explicit P value (e.g. P=0·016 or P=0·32) rather than as a range (e.g. P<0·05 or P>0·05}. It should be stated whether the significance levels quoted are one-sided or two-sided. Where a multiple comparison procedure is used, a description or explicit reference should be given. Where appropriate, a superscript notation may be used in tables to denote levels of significance; similar superscripts should denote lack of a significant difference.

Where the method of analysis is unusual, or if the experimental design is at all complex, further details (e.g. experimental plan, raw data, confirmation of assumptions, analysis of variance tables, etc.) should be included.

Figures. In curves presenting experimental results the determined points should be clearly shown, the symbols used being, in order of preference, ○, ●, Δ, ▲, □, ■, ×, +. Curves and symbols should not extend beyond the experimental points. Scale-marks on the axes should be on the inner side of each axis and should extend beyond the last experimental point. Ensure that lines and symbols used in graphs and shading used in histograms are large enough to be easily identified when the figure is reduced to fit the printed page.

Figures and diagrams can be prepared using most applications but please do not use the following: cdx, chm, jnb or PDF. All figures should be numbered and legends should be provided. Each figure, with its legend, should be comprehensible without reference to the text and should include definitions of abbreviations. Latin names for unusual species should be included unless they have already been specified in the text. Each figure will be positioned near the point in the text at which it is first introduced unless instructed otherwise.

Refer to a recent copy of the journal for examples of figures.

Plates. The size of photomicrographs may have to be altered in printing; in order to avoid mistakes the magnification should be shown by scale on the photograph itself. The scale with the appropriate unit together with any lettering should be drawn by the author, preferably using appropriate software.

Tables. Tables should carry headings describing their content and should be comprehensible without reference to the text. Tables should not be subdivided by ruled lines. The dimensions of the values, e.g. mg/kg, should be given at the top of each column. Separate columns should be used for measures of variance (SD, SE etc.), the ± sign should not be used. The number of decimal places used should be standardized; for whole numbers 1·0, 2·0 etc. should be used. Shortened forms of the words weight (wt) height (ht) and experiment (Expt) may be used to save space in tables, but only Expt (when referring to a specified experiment, e.g. Expt 1) is acceptable in the heading.

Footnotes are given in the following order: (1) abbreviations, (2) superscript letters, (3) symbols. Abbreviations are given in the format: RS, resistant starch. Abbreviations appear in the footnote in the order that they appear in the table (reading from left to right across the table, then down each column). Abbreviations in tables must be defined in footnotes. Symbols for footnotes should be used in the sequence: *†‡§‖¶, then ** etc. (omit * or †, or both, from the sequence if they are used to indicate levels of significance).

For indicating statistical significance, superscript letters or symbols may be used. Superscript letters are useful where comparisons are within a row or column and the level of significance is uniform, e.g. '^{a,b,c}Mean values within a column with unlike superscript letters were significantly different (P<0·05)'. Symbols are useful for indicating significant differences between rows or columns, especially where different levels of significance are found, e.g. 'Mean values were significantly different from those of the control group: *P<0·05, **P<0·01, ***P<0·001'. The symbols used for P values in the tables must be consistent.

Tables should be placed at the end of the text. Each table will be positioned near the point in the text at which it is first introduced unless instructed otherwise.

Please refer to a recent copy of the journal for examples of tables.

Chemical formulas. These should be written as far as possible on a single horizontal line. With inorganic substances, formulas may be used from first mention. With salts, it must be stated whether or not the anhydrous material is used, e.g. anhydrous $CuSO_4$, or which of the different crystalline forms is meant, e.g. $CuSO_4.5H_2O$, $CuSO_4.H_2O$.

Descriptions of solutions, compositions and concentrations. Solutions of common acids, bases and salts should be defined in terms of molarity (M), e.g. 0·1 M-NaH_2PO_4. Compositions expressed as mass per unit mass (w/w) should have values expressed as ng, µg, mg or g per kg; similarly for concentrations expressed as mass per unit volume (w/v), the denominator being the litre. If concentrations or compositions are expressed as a percentage, the basis for the composition should be specified (e.g. % (w/w) or % (w/v) etc.). The common measurements used in nutritional studies, e.g. digestibility, biological value and net protein utilization, should be expressed as decimals rather than as percentages, so that amounts of available nutrients can be obtained from analytical results by direct multiplication. See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences*. London: The Royal Society, 1972 (para. 8).

Nomenclature of vitamins. Most of the names for vitamins and related compounds that are accepted by the Editors are those recommended by the IUNS Committee on Nomenclature. See *Nutrition Abstracts and Reviews* (1978) **48**A, 831–835.

Acceptable name	*Other names**
Vitamin A	
Retinol	Vitamin A_1
Retinaldehyde, retinal	Retinene
Retinoic acid (all-*trans* or 13-*cis*)	Vitamin A_1 acid
3-Dehydroretinol	Vitamin A_2
Vitamin D	
Ergocalciferol, ercalciol	Vitamin D_2 calciferol
Cholecalciferol, calciol	Vitamin D_3
Vitamin E	
α-, β- and γ-tocopherols plus tocotrienols	
Vitamin K	
Phylloquinone	Vitamin K_1
Menaquinone-n (MK-n)†	Vitamin K_2
Menadione	Vitamin K_3, menaquinone, menaphthone
Vitamin B_1	
Thiamin	Aneurin(e), thiamine
Vitamin B_2	
Riboflavin	Vitamin G, riboflavine, lactoflavin
Niacin	
Nicotinamide	Vitamin PP
Nicotinic acid	
Folic Acid	
Pteroyl(mono)glutamic acid	Folacin, vitamin B_c or M
Vitamin B_6	
Pyridoxine	Pyridoxol
Pyridoxal	
Pyridoxamine	
Vitamin B_{12}	
Cyanocobalamin	

Hydroxocobalamin	Vitamin B_{12a} or B_{12b}
Aquocobalamin	
Methylcobalamin	
Adenosylcobalamin	
Inositol	
Myo-inositol	*Meso*-inositol
Choline	
Pantothenic acid	
Biotin	Vitamin H
Vitamin C	
Ascorbic acid	
Dehydroascorbic acid	

*Including some names that are still in use elsewhere, but are not used by the *British Journal of Nutrition*.

†Details of the nomenclature for these and other naturally-occurring quinones should follow the Tentative Rules of the IUPAC-IUB Commission on Biochemical Nomenclature (see *European Journal of Biochemistry* (1975) **53**, 15–18).

Generic descriptors. The terms **vitamin A**, **vitamin C** and **vitamin D** may still be used where appropriate, for example in phrases such as 'vitamin A deficiency', 'vitamin D activity'.

Vitamin E. The term **vitamin E** should be used as the descriptor for all tocol and tocotrienol derivatives exhibiting qualitatively the biological activity of α-tocopherol. The term **tocopherol**s should be used as the generic descriptor for all methyl tocols. Thus, the term **tocopherol** is not synonymous with the term **vitamin E**.

Vitamin K. The term **vitamin K** should be used as the generic descriptor for 2-methyl-1,4-naphthoquinone (menaphthone) and all derivatives exhibiting qualitatively the biological activity of phylloquinone (phytylmenaquinone).

Niacin. The term **niacin** should be used as the generic descriptor for pyridine 3-carboxylic acid and derivatives exhibiting qualitatively the biological activity of nicotinamide.

Vitamin B_6. The term **vitamin B_6** should be used as the generic descriptor for all 2-methylpyridine derivatives exhibiting qualitatively the biological activity of pyridoxine.

Folate. Due to the wide range of C-substituted, unsubstituted, oxidized, reduced and mono- or polyglutamyl side-chain derivatives of pteroylmonoglutamic acid that exist in nature, it is not possible to provide a complete list. Authors are encouraged to use either the generic name or the correct scientific name(s) of the derivative(s), as appropriate for each circumstance.

Vitamin B_{12}. The term **vitamin B_{12}** should be used as the generic descriptor for all corrinoids exhibiting qualitatively the biological activity of cyanocobalamin. The term **corrinoid**s should be used as the generic descriptor for all compounds containing the corrin nucleus and thus chemically related to cyanocobalamin. The term **corrinoid** is not synonymous with the term **vitamin B_{12}**.

Vitamin C. The terms **ascorbic acid** and **dehydroascorbic acid** will normally be taken as referring to the naturally-occurring L-forms. If the subject matter includes other optical isomers, authors are encouraged to include the L- or D- prefixes, as appropriate. The same is true for all those vitamins which can exist in both natural and alternative isomeric forms.

Amounts of vitamins and summation. Weight units are acceptable for the amounts of vitamins in foods and diets. For concentrations in biological tissues, SI units should be used; however, the authors may, if they wish, also include other units, such as weights or international units, in parentheses.

See *Metric Units, Conversion Factors and Nomenclature in Nutritional and Food Sciences* (1972) paras 8 and 14–20. London: The Royal Society.

Nomenclature of fatty acids and lipids. In the description of results obtained for the analysis of fatty acids by conventional GLC, the shorthand designation proposed by Farquhar JW, Insull W, Rosen P, Stoffel W & Ahrens EH (*Nutrition Reviews* (1959), **17**, Suppl.) for individual fatty acids should be used in the text, tables and figures. Thus, 18 : 1 should be used to represent a fatty acid with eighteen carbon atoms and one double bond; if the position and configuration of the double bond is unknown. The shorthand designation should also be used in the abstract. If the positions and configurations of the double bonds are known, and these are important to the discussion, then a fatty acid such as linoleic acid may be referred to as *cis*-9,*cis*-12-18 : 2 (positions of double bonds related to the carboxyl carbon atom 1). However, to illustrate the metabolic relationship between different unsaturated fatty acid families, it is sometimes more helpful to number the double bonds in relation to the terminal methyl carbon atom, *n*. The preferred nomenclature is then: 18 : 3*n*-3 and 18 : 3*n*-6 for α-linolenic and γ-linolenic acids respectively; 18 : 2*n*-6 and 20 : 4*n*-6 for linoleic and arachidonic acids respectively and 18 : 1*n*-9 for oleic acid. Positional isomers such as α- and γ-linolenic acid should always be clearly distinguished. It is assumed that the double bonds are methylene-interrupted and are of the *cis*-configuration (see Holman RT in *Progress in the Chemistry of Fats and Other Lipids* (1966) vol. 9, part 1, p. 3. Oxford: Pergamon Press). Groups of fatty acids that have a common chain length but vary in their double bond content or double bond position should be referred to, for example, as C_{20} fatty acids or C_{20} PUFA. The modern nomenclature for glycerol esters should be used, i.e. triacylglycerol, diacylglycerol, monoacylglycerol *not* triglyceride, diglyceride, monoglyceride. The form of fatty acids used in diets should be clearly stated, i.e. whether ethyl esters, natural or refined fats or oils. The composition of the fatty acids in the dietary fat and tissue fats should be stated clearly, expressed as mol/100 mol or g/100 g total fatty acids.

Nomenclature of micro-organisms. The correct name of the organism, conforming with international rules of nomenclature, should be used: if desired, synonyms may be added in parentheses when the name is first mentioned. Names of bacteria should conform to the current Bacteriological Code and the opinions issued by the International Committee on Systematic Bacteriology. Names of algae and fungi must conform to the current International Code of Botanical Nomenclature. Names of protozoa should conform to the current International Code of Zoological Nomenclature.

Nomenclature of plants. For plant species where a common name is used that may not be universally intelligible, the Latin name in italics should follow the first mention of the common name. The cultivar should be given where appropriate.

Ethics of human experimentation. The notice of contributors is drawn to the guidelines in the World Medical Association (2000) Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects, with notes of clarification of 2002 and 2004 http://www.wma.net/e/policy/b3.htm, the *Guidelines on the Practice of Ethics Committees Involved in Medical Research Involving Human Subjects* (3rd ed., 1996; London: The Royal College of Physicians) and the Guidelines for the Ethical Conduct of Medical Research Involving Children, revised in 2000 by the Royal College of Paediatrics and Child Health: Ethics Advisory Committee (*Arch Dis Child* (2000) **82**, 177–182). A paper describing any experimental work on human subjects should include a statement that ethical approval has been obtained.

Animal experimentation. The Editors will not accept papers reporting work carried out using inhumane procedures. Authors should indicate that their experiments have been approved by the appropriate local or national ethics committee for animal experiments.

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ADDENDUM C: INFORMED CONSENT FORM

BeForMi Informed Consent Form and Information

Dear Parent / legal guardian

Re: INFORMATION about the BeForMi study and request for informed consent

The North West University wants to undertake a research study to determine the effect of a flavoured cold drink that contains vitamins and minerals (BeForMi) on the learning ability in primary school learners in grades 1 to 3 at the school where your child is enrolled. Research studies that have been done in the past have shown that additional vitamins and minerals may improve a child's ability to learn. We have designed 4 different cold drinks, some with vitamins and some without, to enable us to find out if these vitamins and minerals can improve your child's learning ability. We have planned a study in which the children will be given 200ml of BeForMi each day before break for the duration of the whole year. Your child may receive any one of the four. We are asking for your consent for your child to take part in this study. Participation is completely voluntarily and the child can drop out of the study at any time.

The study will involve the following:

1. We want to take a picture of each child to help the study assistants recognise the children in the study and that we are sure to compare measurements of the same child before and after the study.
2. Your child's height, weight, arm circumference and fat skin-folds will be measured. The skin-fold measurements are taken by using a machine that folds the skin in order to measure how thick it is.
3. To measure your child's ability to learn, your child will be asked questions in a personal interview by a trained adult assistant.
4. Your child will be asked to step on a machine which looks like a scale and some measurements will be taken. These measurements will be used to calculate how much fat and muscle the child has in the body.

5. For us to know how much nutrients the child has we will need to take a small blood sample (about two teaspoons). The blood samples will be taken by an experienced registered nurse who normally does this type of work. She will use sterile equipment (only used once and then discard it).
6. All the measurements will be taken once at the beginning of the study and again at the end of the school year.
7. During the study period you will be visited at your home by a study assistant. The assistant will interview you about the food you ate the previous day and also ask about your general living conditions.

Benefits of the study to your child:

1) Every day all children will receive a cold drink as part of their meal at school during the 2010 school year.
2) After the study has finished all learners in the school will be given the drink that has been shown to improve their learning ability to the greatest extent for the first half of the 2011 school year.
3) Children who are found to be severely anaemic when we test the blood will be identified confidentially and your will be referred to the clinics so that the child can receive treatment immediately.
4) The children will be given de-worming medication before the study. This will ensure that the children may benefit from the micronutrients they get.
5) All the utensils that will be used to serve the children with the drinks will be left at the school for the sole use of the school feeding programme.

If you have any questions please feel free to phone

Dr. Namukolo Covic 018 299 4037 or 072 443 6895

Professor Johann Jerling 018 299 2481

Yours Sincerely,

Dr. Namukolo Covic

INFORMED CONSENT FORM

Effect of long-term consumption of a beverage fortified with vitamins and minerals on the learning ability of primary school children aged 7-9 years in North-West Province of South Africa: the BeForMi study

I have been informed about the purpose and nature of the study and that all information will be regarded as confidential.

I have been informed about the advantages and possible adverse effects that may result from procedures and/or treatment, and I understand what it says.

I understand that participation is voluntary and that I can recall my consent at anytime without forfeiting the availability of any future routine medical care.

Nutritional status will be assessed by means of the measurement of height, weight, skinfold measurements, body composition measurement and analysis of blood samples. A small blood sample of about two teaspoons will be taken from the child's arm by a nursing sister at the beginning of the year and end of the study at the end of the year.

Name of child …….... My child is allergic to:

……

Date of birth of child: Day …..…… Month……..…….. Year ……..

The gender of the child Male ☐ Female ☐ *please tick one as approriate*

Residential address……

……
……

Name of Parent or Legal Guardian ……. Signiture:…….

Signed this…….. Day Of …… 2010 at ……..

Telephone number of Name of Parent or Legal Guardian